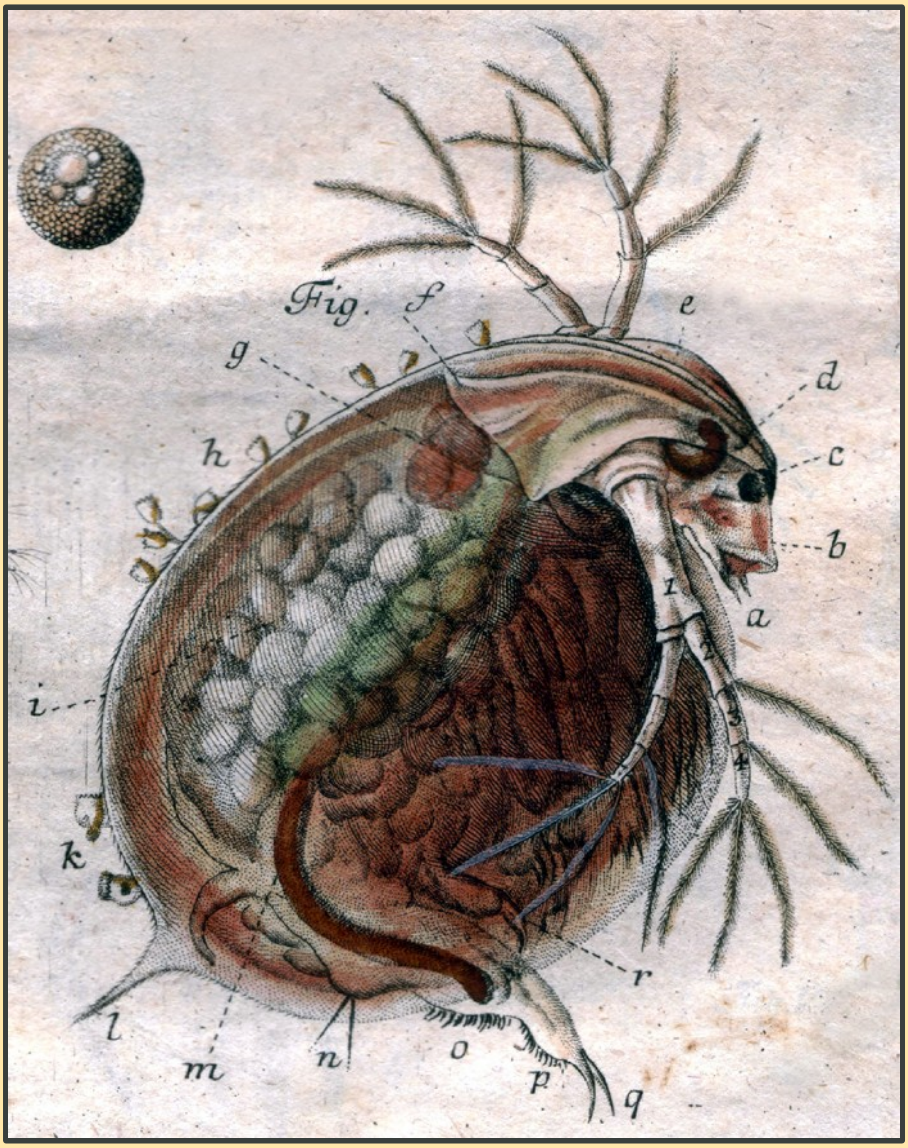
Ecology, Epidemiology and Evolution of Parasitism in *Daphnia*



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The cover illustration is part of a drawing from the monograph "Die günen Armpolypen, die geschwänzten und ungeschwänzten zackigen Wasserflöhe und eine besondere Art kleiner Wasseraale" by Jacob Christian Schaffer, published in Regensburg, Germany in 1755. The picture shows the external and internal anatomy of *Daphnia magna*. Note that on the dorsal side the female carries numerous peritrich (Ciliata) epibionts. In the upper left corner a parthenogenetic egg is drawn.

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Contents

Acknowledgments

In 1990, I was working on my PhD on the life-history evolution of *Daphnia* at the Zoological Institute of the University of Basel when Bill Hamilton came for a visit there. Being deeply interested in the evolution of parasites, he did not wait long before asking me about parasites in *Daphnia*. To my embarrassment, I had to answer that I had never seen a parasite in *Daphnia* and that I believed they may not be very important. Of course Bill was not convinced by my obviously rather superficial and uninformed statement, and after some discussion I agreed that I would collect some field samples the next day and screen them with him for parasites. Except for a few individual *Daphnia* carrying some algae epibionts, we did not find anything. Bill was convinced, however, that there was more to discover, and a month later he sent me a copy of Green's 1974 paper on "Parasites and Epibionts of Cladocera." Green lists numerous symbionts living on and in *Daphnia* and other Cladocerans—too many to be ignored.

At the same time, Paul Schmid-Hempel's group was working at the Zoological Institute in Basel, establishing bumblebees as a new model system to study host–parasite interactions. On the basis of many interactions with Paul and his students, mainly Jacqui Shykoff and Christine Müller, I started to develop an interest in host–parasite evolution and eventually decided that this may be a good topic for a postdoc. Interestingly, other PhD students in our group pursued the same direction.

Being an experimentalist at heart, I decided that I would like to start working with a rather simple host–parasite system that would allow me to do sophisticated experiments. I asked Bill Hamilton to host me in Oxford. Bill was enthusiastic, but we were undecided about what system would be good. Bill suggested *Daphnia*, but I was not so sure, because I still had not seen parasites in *Daphnia*. The advantage with *Daphnia,* however, was that I already had experience with them and that they seemed, indeed, like a good system: easy to maintain, reproducing clonally, short generation time, and small size, to name just a few. Nonetheless, before I settled on *Daphnia,* I did an intensive literature search (back in the early nineties, this was still a time-consuming enterprise) and came up with three host systems I wanted to check out: the Indian meal moth (*Plodia interpunctella*), *Tribolium*, and *Daphnia*. The two earlier systems are genetically more tractable than *Daphnia,* because genetic crosses of *Daphnia* clones are still not very easy. In the end, one factor swayed my decision to work with *Daphnia*: How could one control airborne pathogens during experiments in which controls and infected replicates are standing side by side? With waterborne pathogens, I reasoned, this would be much less of a problem. It turned out to be correct.

My initial field surveys around Oxford were not very successful: plenty of *Daphnia*, but no parasites. After several months, I decided to contact Jim Green, who was still working in London but was about to retire. He invited me to bring him the samples in which I wanted to find parasites. It took him 10 minutes to find the first parasites, and over the next two hours a few more species turned up as well. What I did not know was that it is essential to use phase-contrast microscopy, because microsporidian parasites, in particular, are barely visible without it. Despite his enthusiasm, Jim was rather pessimistic about the possibilities of culturing *Daphnia* parasites and conducting controlled experiments. However, back in Oxford I checked all of my cultures and realized that I had already cultured one species for several months together with the *D. magna* clone it lived in. Within 2 weeks I worked out a method for controlled culturing of *Glugoides intestinalis* (formerly *Pleistophora intestinalis*) and 3 months later submitted the first paper

on it. Since then, my lab has developed culturing methods for more than 10 species of parasites.

This work with the *Daphnia* parasites has absorbed my attention since then, and together with my research group, I have spent many exciting hours in the laboratory and in the field finding out more about *Daphnia* parasites, and about parasites in general. The *Daphnia*–microparasite system has proved to be a powerful model for many questions in basic epidemiology, ecology, and evolution. The upcoming genome data will extend this into the "Narrow Roads of Gene Land" (the title of Bill Hamilton's second book with many parts on the evolution of host–parasite interactions, 2001) and will hopefully open up a pluralistic approach to understanding host–parasite coevolution.

This book would not have been possible without the collaboration of the marvelous people working with me in Oxford, Silwood, Basel, and Fribourg, and also collaborators from around the world who shared my enthusiasm for host–parasite interactions. I want to thank the people in and around my group (in roughly chronological order): Valentino Lee, Katrina Mangin, Heide Stirnadel, Sven Krackow, Judy Wearing-Wilde (Oxford), Dermot McKee (Silwood Park), Christine Zschokke-Rohringer, Hans-Jochaim Carius, Tom Little, Daniel Fels, Marc Capaul, Myriam Riek, Patrick Mucklow, Pia Salathé, Katja Pulkkinen (Basel), Christoph Haag, Dominik Refardt, Dita Vizoso, Olga Sakwinska (Basel and Fribourg), Lusia Sygnarski, Sandra Lass, Marc Zbinden, Knut Helge Jensen, Raffael Aye, Florian Altermatt, Holly Ganz (Fribourg and Basel), and Thomas Zumbrunn (Basel). Special thanks to Jürgen Hottinger, who became over the years not only a close friend but also the irreplaceable center of the group. Numerous collaborators, many of whom are theoreticians, helped open my eyes when I was blinded by the beauty of *Daphnia* symbionts: Sebastian Bonhoeffer, Marc Lipsitch, Martin Nowak, Wolfgang Weisser, Richard Lenski, Bill Hamilton, Paul Rainey, Mitja Scholz, Martin Embley, Janne Bengtsson, Liz Canning, Steve Stearns, Ilmari Pajunen, Roland Regoes, Ronny Larsson, Kerstin Bittner, Ellen Decaestecker, and Paul Schmid-Hempel. Florian Altermatt, Holly Ganz, Sandra Lass, Dominik Refardt, Marc Zbinden and Thomas Zumbrunn read most of the book in earlier drafts and helped me to polish style and content. Dita Vizoso produced several figures to illustrate important aspects of life cycles and transmission. Dita Vizoso and Frida Ben-Ami are thanked for contributing photographs. I thank my son Gleb for help with producing some of the figures. Suzanne Zweizig improved the style and readability of the language throughout the book. Rita Gunasekera helped me with the formatting of the various files. Jo McEntyre and Laura Dean were of great help in all aspects of publishing this book. Thanks to their work, the book can be made available to a wide audience via the Internet. Finally, I want to thank my friends and family for their support and the encouragement to write this book.

November 2005 Dieter Ebert

Chapter 1

Introduction to the Ecology, Epidemiology, and Evolution of Parasitism in *Daphnia*

This chapter provides a short introduction to the topic of the book. I define parasites as any small organism closely associated with, and harmful to, a larger organism. I point out which features of parasites make them attractive ecological factors and why the study of parasites may add to our understanding of *Daphnia* biology. I also provide a general outline of the book's organization.

1.1 Foreword

In 1974, Jim Green published his excellent review of the "Parasites and Epibionts of Cladocera" (Green 1974). Until now, this has been the key reference in this field, not only for the taxonomy of parasites and epibionts but also for their natural history. The strength of Green's review is that it is a comprehensive account of what was known about parasites and epibionts of Cladocerans at the time. Historically this meant, however, that parasites, and in particular microparasites, were poorly documented, because little was known about these tiny organisms. One of my aims here is to concentrate particularly on the parasites, because their roles in the ecology and the evolution of their hosts have been neglected for a long time. Although my original plan was to keep the framework of this book similar to Green's paper, I soon realized that this was not possible because too much new material is available. As my interest centers more on parasitism, I focus here on parasites, leaving aside epibionts. There is, moreover, so much information about parasitism in the Cladocera that I have decided to split the work into two parts and publish it as two independent books. The present book deals with the ecology, epidemiology, and evolution of the parasites of *Daphnia* and other Cladocerans. The second part will deal with the taxonomy and natural history of all parasites known to *Daphnia*.

1.2 Setting the Stage

The ecology of members of the genus *Daphnia* has possibly been more closely investigated than any other taxon. For centuries, researchers studied *Daphnia* ecology not only for its key role as a primary consumer in the food chain of freshwater ponds and lakes but also as a model species for phenotypic plasticity (e.g., cyclomorphosis and predator-induced defense), behavior (e.g., vertical migration), toxicology, and the evolution of sexual and asexual reproduction (e.g., geographic parthenogenesis). In recent years, a burst of genetic

research has addressed a number of evolutionary questions, resulting in a well-rounded picture of the evolutionary ecology of the genus.

For a long time, research focused on predators as the main enemies of *Daphnia* in their natural habitat. Predatory fish, phantom midge larvae, and water boatmen were among the key culprits and received a lot of attention, in particular after predator-induced defenses were described. This interest in predators existed in sharp contrast to the lack of attention paid to another class of *Daphnia's* natural enemies, parasites and epibionts. Despite numerous taxonomic studies on the epibionts and parasites of *Daphnia,* there was less than a handful of ecological studies on them up until about 15 years ago. The growing awareness that parasites are ubiquitous and may play an important role in most natural ecosystems has changed this, as the increasing number of publications about *Daphnia* parasites and epibionts confirms (Figure 1.1).

1.3 Defining Parasites

Although parasites have traditionally been defined by a combination of conceptual and taxonomic features, I use an entirely conceptual definition here. I consider a parasite to be any small organism (including viruses) that lives in close association with a host organism and for which it seems reasonable to assume that the host carries some cost. These costs may be clearly visible, in the form of reduced fecundity or survival, but may in some cases be subtle. For example, reduced sexual attractiveness (leading to reduced mating success) or reduced competitive ability may not be very visible. I devote an entire chapter to discussing the fitness costs caused by parasites. This conceptual definition of a parasite includes members of various taxa, such as viruses, bacteria, fungi, and protozoa, but also includes functional categories (not taxonomically defined), such as pathogens and helminths. In contrast to typical predators, parasites do not always kill their hosts, and if they do, it may take a considerable amount of time, during which the parasite may be transmitted to other hosts, and the host remains in the community competing with other organisms for space, food, and mating partners.

In the literature on Cladocera and more specifically on *Daphnia,* parasites are often distinguished from epibionts. Whereas the former are usually endoparasites, i.e., located within the body of the host, the latter are located on the body surface and may therefore be labeled as ectoparasites. In the main part of this book, I concentrate on endoparasites and exclude epibionts. However, this is not to say that epibionts are not parasites or are not important. In fact, I believe that most epibionts fulfill the definition of parasites used here, because they are often closely associated with their hosts and cause harm to their hosts. This harm may not be directly visible, but there are certainly increased costs for swimming, which may have consequences for other fitness components, such as fecundity, survival, competition, and mate finding (Threlkeld et al. 1993). It has also been suggested that epibiontic filter feeders compete with their hosts for food (Kankaala and Eloranta 1987). On the other hand, it has been suggested that under certain conditions, high loads of algal epibionts may provide additional food for the host and thus result in a net benefit (Barea-Arco et al. 2001). However, this form of a food supplementation is certainly not the typical effect of epibionts.

I do not include epibionts in this book, because I feel that there is less need to discuss the epidemiology of this functional group than for endoparasites. However, I will refer to them whenever it might further our understanding of *Daphnia*–parasite interactions.

1.4 Host–Parasite Interactions

Parasites may be directly or indirectly involved in the ecology and evolution of a broad range of phenomena: host population dynamics and extinctions, maintenance of genetic diversity, sexual selection, evolution of genetic systems, and evolution of sexual recombination, to name just a few. Certainly, parasites possess features that make them very attractive as explanatory factors in the evolution and ecology of their hosts. These features include their high abundance in nearly every ecosystem, their typically narrow host range (compared with typical predators), their adverse effects on their hosts (e.g., reduced fecundity and survival), and density dependence during horizontal transmission (Anderson 1979, 1993; Anderson and May 1978; May and Anderson 1979; Price 1980).

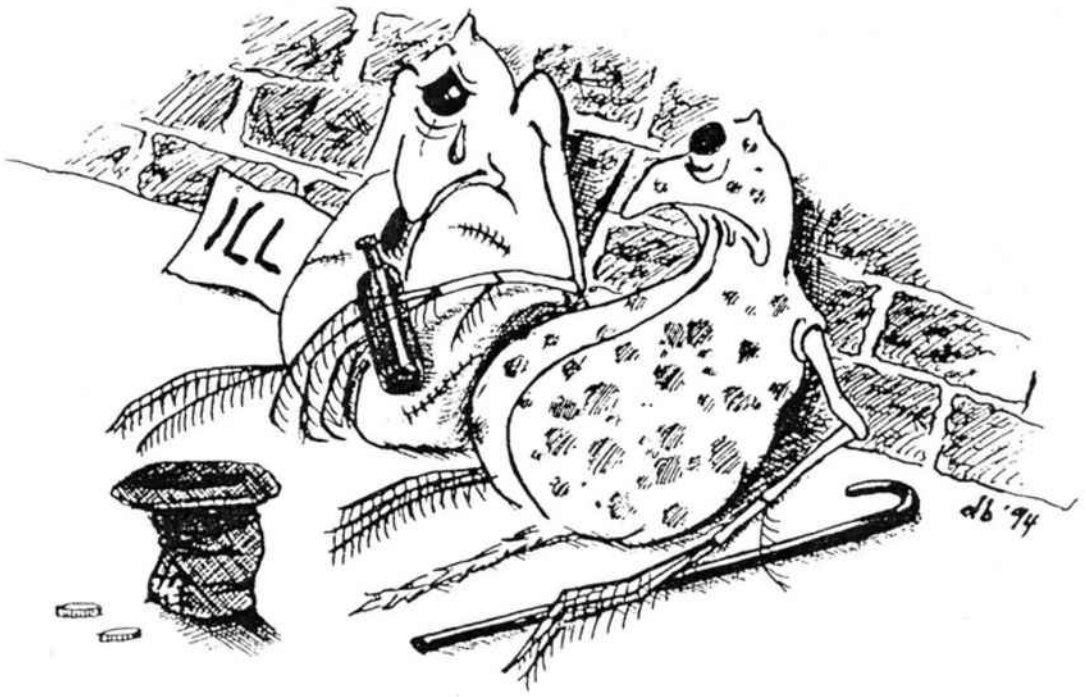

Figure 1.1 The hard life of *Daphnia*. Drawing by Daniela Brunner, Basel.

On the other hand, hosts are the environment for the parasites and thus define their niche. Most parasites are not viable outside of their hosts for extended periods (not considering resting stages) and therefore—from the parasite's point of view—parasite and host form an inseparable biological unit. Thus, parasite ecology is closely linked to the ecology of its hosts, and the parasite's natural history is best seen in the light of its host's biology. In this book, I focus largely on members of the genus *Daphnia* as hosts. Whenever possible, I include information on other Cladocerans.

1.5 Outline of This Book

Following this introduction, Chapter 2 gives a general summary of *Daphnia* biology, highlighting at the same time those aspects that may be relevant for the study of parasitism. This summary is followed by Chapter 3, which introduces the reader to certain parasite species that are frequently mentioned in the book. This chapter is short, however, because the parasites will be dealt with in detail in the second book.

The next chapters describe the interactions between parasites and their *Daphnia* hosts. Much of the conceptual parts are derived from general principles of epidemiology but with special reference to the biology of zooplankton and especially *Daphnia*. Chapter 4 summarizes what we know from parasitological field studies of Cladocerans. Chapters 5 and 6 deal with the sometimes severe fitness consequences of parasitism. In Chapter 5, I review what we know about the negative effect of parasites on the fitness of individual hosts, and in Chapter 6, I review the little we know about how hosts fight parasites. Chapter 7 is on host ranges and discusses what we know about the specificity of *Daphnia* parasites.

Chapters 8 and 9 address aspects of parasitism at the population level. A central chapter of this book is "Epidemiology." Its two parts deal with transmission processes and with the actual epidemiology of *Daphnia* parasites. Chapter 9 introduces the important question of whether parasites regulate their host populations or even drive them to extinction.

Chapter 10 introduces a number of experiments that one may do with *Daphnia* and its parasites within the framework of a student course or for research purposes. These simple experiments may be used to illustrate principles of host–parasite interactions. Experiments are suggested at the individual level as well as at the population level.

From Chapter 4 onward, I end each chapter by posing open questions and highlighting major gaps in our knowledge.

A Glossary provides definitions of terms from *Daphnia* biology and parasitology used throughout the book.

1.6 Updates and Corrections

I will maintain a Web site on my home institution's server to report updates and correct errors. If you find errors, disagree with certain statements, or find that I neglected important information, I would be happy to read your comments. Please send me an email: dieter.ebert@unibas.ch

Chapter 2

Introduction to *Daphnia* Biology

This chapter provides an overview of the biology of waterfleas of the genus *Daphnia*. It describes basic aspects of individual physiology and nutrition, including some remarks about immunity. It summarizes the typical life cycle and development of *Daphnia*. The modes of reproduction and the induction of resting egg production in cyclic and obligate parthenogenetic forms are discussed. Finally, population biological aspects, such as behavioral ecology, habitat preferences, population genetics, and population dynamics, are introduced.

2.1 Introduction

The following introduction summarizes the key aspects of the natural history of the genus *Daphnia*. It is far from exhaustive. Because much of the biology has been studied over the past 250 years and is considered to be common knowledge, I give no references to specific studies in most cases. The interested reader may consult Freyer (1991), Kästner (1993), Lampert and Sommer (1999), and Peters and De Bernardi (1987), from which much of the information was taken. A search on the Internet using the term "Daphnia" or "Cladocera" will also bring up a large body of information. Of particular interest is the Cladocera Web page of the University of Guelph in Canada. The reader familiar with *Daphnia* may skip this chapter and move on to Chapter 3.

Daphnia are planktonic crustaceans that belong to the Phyllopoda (sometimes called Branchiopoda), which are characterized by flattened leaf-like legs used to produce a water current for the filtering apparatus. Within the branchiopods, *Daphnia* belong to the Cladocera, whose bodies are enclosed by an uncalcified shell (Figures 2.1 and 2.2), known as the carapace. It has a double wall, between which hemolymph flows and which is part of the body cavity. The carapace is largely made of chitin, a polysaccharide. Cladocera have up to 10 pairs of appendages, which are (from front to back): antennules, antennae (the second antennae, used for swimming); maxillae; and mandibles; followed by 5 (as in *Daphnia*) or 6 limbs on the trunk. The limbs form an apparatus for feeding and respiration. At the end of the abdomen is a pair of claws. The body length of Cladocera ranges from less than 0.5 mm to more than 6 mm. Males are distinguished from females by their smaller size, larger antennules, modified post-abdomen, and first legs, which are armed with a hook used in clasping.

The genus *Daphnia* includes more than 100 known species of freshwater plankton organisms found around the world (see Figures 2.3, 2.4, and 2.5 for three European representatives of the genus). They inhabit most types of standing freshwater except for extreme habitats, such as hot springs. All age classes are good swimmers and are mostly pelagic, i.e., found in the open water. They live as filter feeders, but some species may frequently be seen clinging to substrates such as water plants or even browsing over the bottom sed-

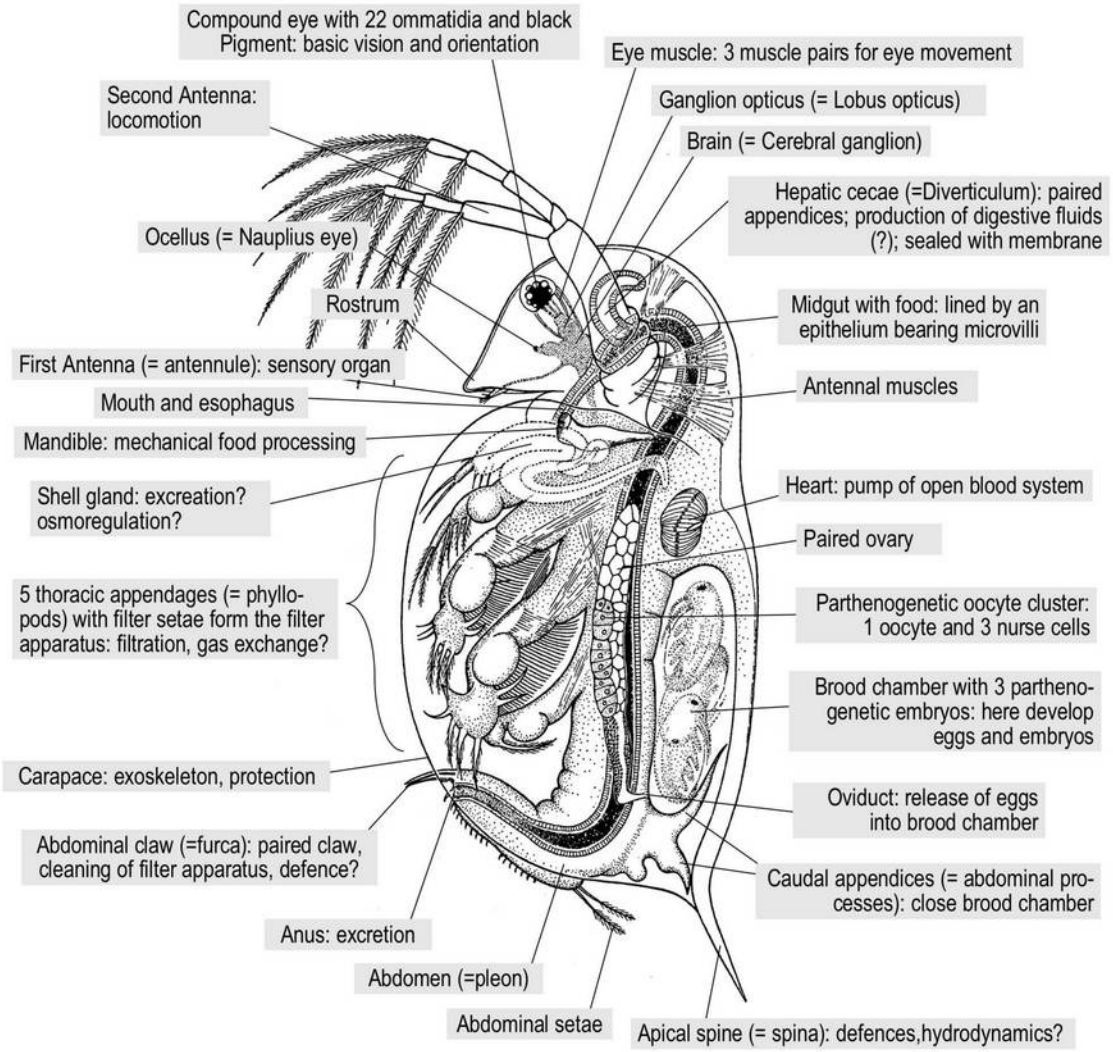

Figure 2.1 The functional anatomy of *Daphnia*. This drawing shows an adult female with parthenogenetic embryos in her brood chamber. For better illustration, the carapace is shown as transparent. The animal measures about 2 mm from the top of its head to the base of its tail spine. Modified after Matthes (first published on page 154 in Kükenthal and Matthes 1944) (with permission from Kästner: Lehrbuch der speziellen Zoologie, Band 1, Teil 4, 1993 © Elsevier GmbH, Spektrum Akademischer Verlag, Heidelberg). Compare this figure with Figure 2.2.

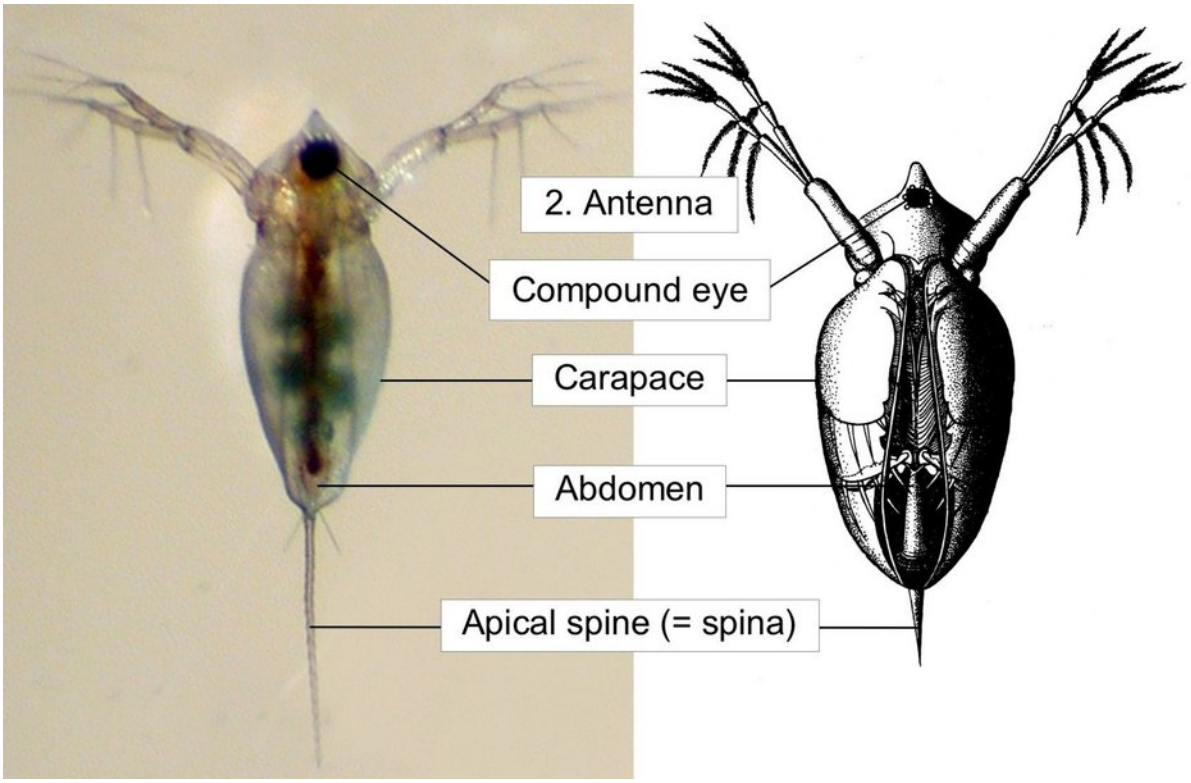

Figure 2.2 Ventral view of *Daphnia* Adult females. On the *left*, a photograph of *Daphnia longispina* from a rock pool population in southern Finland. On the *right*, a drawing. (The drawing is reproduced with permission from Kästner: Lehrbuch der speziellen Zoologie, Band 1, Teil 4, 1993 © Elsevier GmbH, Spektrum Akademischer Verlag, Heidelberg).

iments of shallow ponds. Adults range from less than 1 mm to 5 mm in size, with the smaller species typically found in ponds or lakes with fish predation. The ecology of the genus *Daphnia* may be better known than the ecology of any other group of organisms.

2.2 Physiology, Metabolism, and Immunity

Daphnia feed on small, suspended particles in the water. They are suspension feeders (filter feeders). The food is gathered with the help of a filtering apparatus, consisting of the phylopods, which are flattened leaf-like legs that produce a water current. As the current flows anterior to posterior, the *Daphnia* collect particles that are transferred into the food groove by special setae. Although the feeding apparatus is so efficient that even bacteria can be collected, the food is usually made up of planktonic algae. Green algae are among the best food, and most laboratory experiments are done with either *Scenedesmus* or *Chlamydomonas*, both of which are easy to culture in monoclonal chemostats. *Daphnia* usually consume particles from around 1 μm up to 50 μm, although particles of up to 70 μm in diameter may be found in the gut content of large individuals.

The dynamics of food uptake follow a functional response type 1. Below a certain food concentration (the incipient limiting level), the food uptake from the water (feeding rate) is proportional to the food concentration, and the filtering rate (amount of water filtered per unit time) is maximal. Above this level, the feeding rate is constant because the filtering rate decreases with increasing food concentration in the water. For parasites that enter the

Figure 2.3 ***Daphnia magna***. Adult female with a clutch of freshly laid parthenogenetic eggs in its brood chamber. The female is from a laboratory culture of a clone originating in a pond near Oxford, UK.

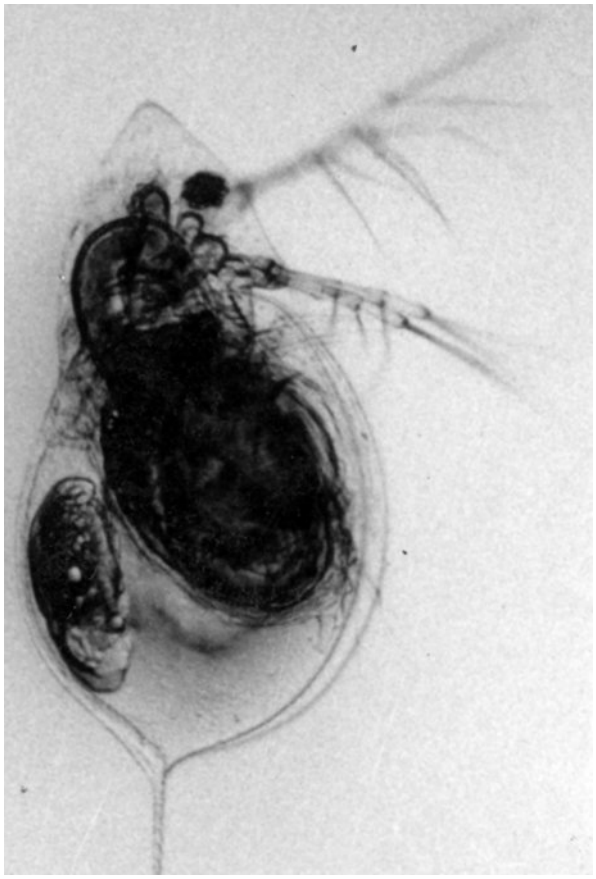

Figure 2.4 ***Daphnia cucullata***. Adult female with one embryo in her brood chamber. This female comes from a laboratory culture of a clone originally isolated from Klostersee in southern Bavaria, Germany.

host with the food particles, infection rates depend on the food concentration in the water. Highest infection rates are expected when filtering rates are maximal.

The gut is more or less tubular with three parts: the esophagus, the midgut, and the hindgut. There are two small digestive ceca (diverticula) that are easily seen in the head section of the midgut (Figures 2.1 and 2.6). The midgut is lined with an epithelium and bears microvilli. Peristaltic contractions of the gut wall pass food through the gut, but a peritrophic membrane contains the food and prevents it from entering the ceca. Epithelial cells do not phagocytose particles but absorb molecules. The pH is 6 to 6.8 in the anterior part of the midgut and 6.6 to 7.2 in the posterior part. Food is expelled from the hindgut by peristaltic movement but also requires the pressure of more recently acquired food particles. The color of *Daphnia* adapts to the food that is predominant in their diet. *Daphnia* feeding on green algae will be transparent with a tint of green or yellow, whereas those feeding on bacteria will be white or salmon-pink. Well-fed animals are more strongly colored than starved animals.

Daphnia have an open blood circulation. The heart is located dorsally and anterior from the brood chamber. At 20°C, it beats about 200 times per minute, slowing down at lower temperatures. Blood cells are easily visible through the transparent body as they flow rapidly through the body cavity. To support oxygen transport,

Figure 2.5 ***Daphnia longispina***. Adult female with three eggs in the brood chamber. This female was isolated from a rock pool in southern Finland close to Tvärminne.

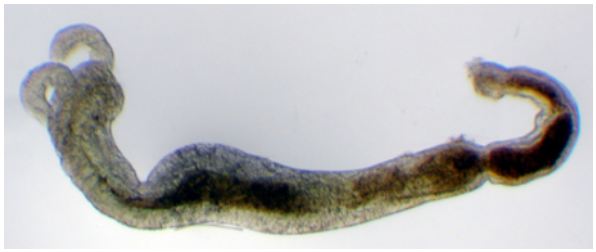

Figure 2.6 Gut of *Daphnia magna*. Gut dissected from a female. On the *left*, the paired intestinal ceca can be seen. The gut ends at the *right* side. The esophagus cannot be seen in this preparation. The dark material is partially digested gut content.

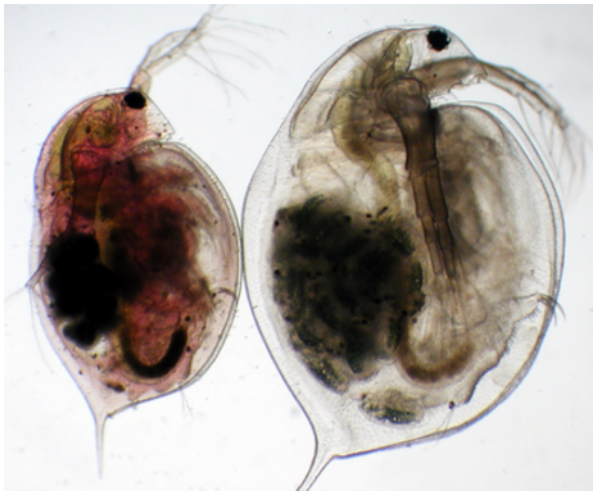

Figure 2.7 Two *Daphnia magna* with contrasting hemolymph color due to haemoglobin (Hb). These two adult females were taken from two adjacent rock pool populations in southern Finland at a time when one of the pools was low in oxygen (because of overfertilization from bird droppings). In the *left* female, low oxygen triggered the production of Hb, which gives the hemolymph a reddish color. The female on the *right* was taken from a pool with clear water and apparently normal oxygen levels. The size difference of the animals is attributable to the arbitrary choice of specimens. Both females are carrying embryos in their brood chambers.

Daphnia have the extracellular respiratory protein hemoglobin (Hb), a multi-subunit, multi-domain macromolecule. There are at least four Hb genes. *Daphnia* tend to develop more Hb to increase oxygen uptake from the water. In response to environmental changes (oxygen concentration, temperature), the Hb concentration varies up to about 20-fold. Oxy-hemoglobin, the form that is loaded with oxygen, is red and gives the transparent animals a reddish appearance (Figure 2.7). Because certain parasites also cause the hemolymph to become red, one cannot easily determine the cause of the red color from sight alone. However, low oxygen usually affects an entire population, coloring all animals reddish, whereas parasites usually infect only a portion of the population.

Daphnia have the usual osmoregulatory problems of freshwater animals, i.e., too much water and too few solutes. They are able to absorb ions with chloride-absorbing glands. The shell gland (maxillary gland; Figure 2.1) may have a role in excretion and/or osmoregulation.

The nervous system is characterized by the cerebral ganglion, which is located close to the gut and near the eye. Juvenile and adult *Daphnia* have one large compound eye, whereas embryos show two brownish eye spots that fuse during the last part of the development. The compound eye helps to orient the animal while swimming. A small structure

called a naupliar eye is located between the mouth and the compound eye on top of the cerebral ganglion.

Many invertebrates, including crustaceans, have a well-developed innate immune system that includes melanization by activation of the prophenoloxidase (proPO) activating system, a clotting process, phagocytosis, encapsulation of foreign material, antimicrobial action, and cell agglutination (Söderhall 1999). Of these mechanisms, only the proPO system (Mucklow and Ebert 2003) and phagocytosis (Metchnikoff 1884) have been identified in *Daphnia*, although there is no reason to doubt that the other mechanisms work as well. The proPO system is not only a defense system against parasites but is also involved in wound healing of the cuticle (Figure 2.8). The enzyme involved in melanin formation, PO, has been detected in the blood of many arthropods, including *D. magna*. Melanin is a brown pigment that is also used for carapace pigmentation in some *Daphnia*, particularly *Daphnia* in the high Arctic, where the dark pigment protects them from uninterrupted solar radiation (repair of UV damage is only possible in the dark). Acquired immunity is thought to be absent in invertebrates; however, transmission of strain-specific immunity from mothers to offspring has recently been suggested for *D. magna* (Little et al. 2003).

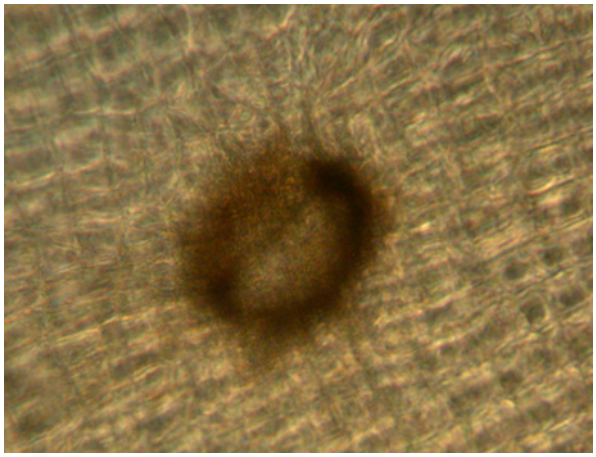

Figure 2.8 Wound healing in *Daphnia magna*. An injury in the carapace heals within a few hours to days. Wound healing involves a melanization reaction that stains the wound dark. Here a needle was used to injure the carapace. The squared pattern in the background shows the epidermal cell structure.

2.3 Life Cycle and Development

The life cycle of *Daphnia* during the growth season is characterized by its asexual mode of reproduction (apomixis) (Figure 2.9). A female produces a clutch of parthenogenetic (amictic) eggs after every adult molt (if feeding conditions permit). Figures 2.3 to 2.5 show females with parthenogenetic eggs. The eggs are placed in the brood chamber, which is located dorsally beneath the carapace and which is closed by the abdominal processes (Figures 2.1 and 2.10). Development of eggs is direct (immediate). At 20°C, the embryos hatch from the eggs after about 1 day but remain in the brood chamber for further development (Figure 2.11). After about 3 days in the brood chamber, the young *Daphnia* are released by the mother through ventral flexion of the post-abdomen. The newborn look more or less like the adult *Daphnia*, except that the brood chamber is not yet developed (Figure 2.12). In most species, a juvenile *Daphnia* passes through four to six juvenile instars before it becomes primipare, i.e., produces eggs for the first time. The age at which the first eggs are deposited into the brood chamber is around 5-10 days at 20°C, but this may take longer under poor feeding conditions. An adult female may produce a clutch of eggs every 3 to 4 days until her death. In the laboratory, females may live for more than 2 months, with a higher age being reached under poorer feeding conditions. Clutch sizes vary among species, from 1 to 2 eggs in small species such as *D. cucullata* (Figure 2.4) to more than 100 in large species such as *D. magna* (Figure 2.3).

Although in a typical growth season *Daphnia* produce diploid (2N) eggs that develop directly and without a resting phase, a different type of egg is produced for resting (Figure 2.9). These resting eggs are encapsulated in a protective, saddle-like structure called an ephippium (Figures 2.13 and 2.14), which is usually strongly melanized and contains 2 large eggs, 1 from each ovary. It is not uncommon, however, to find ephippia with only 1 egg, or none at all. The ephippium is cast off at the next molt. In most cases, these eggs are produced sexually, but obligate parthenogenetic *Daphnia*, which are typically found in the north-

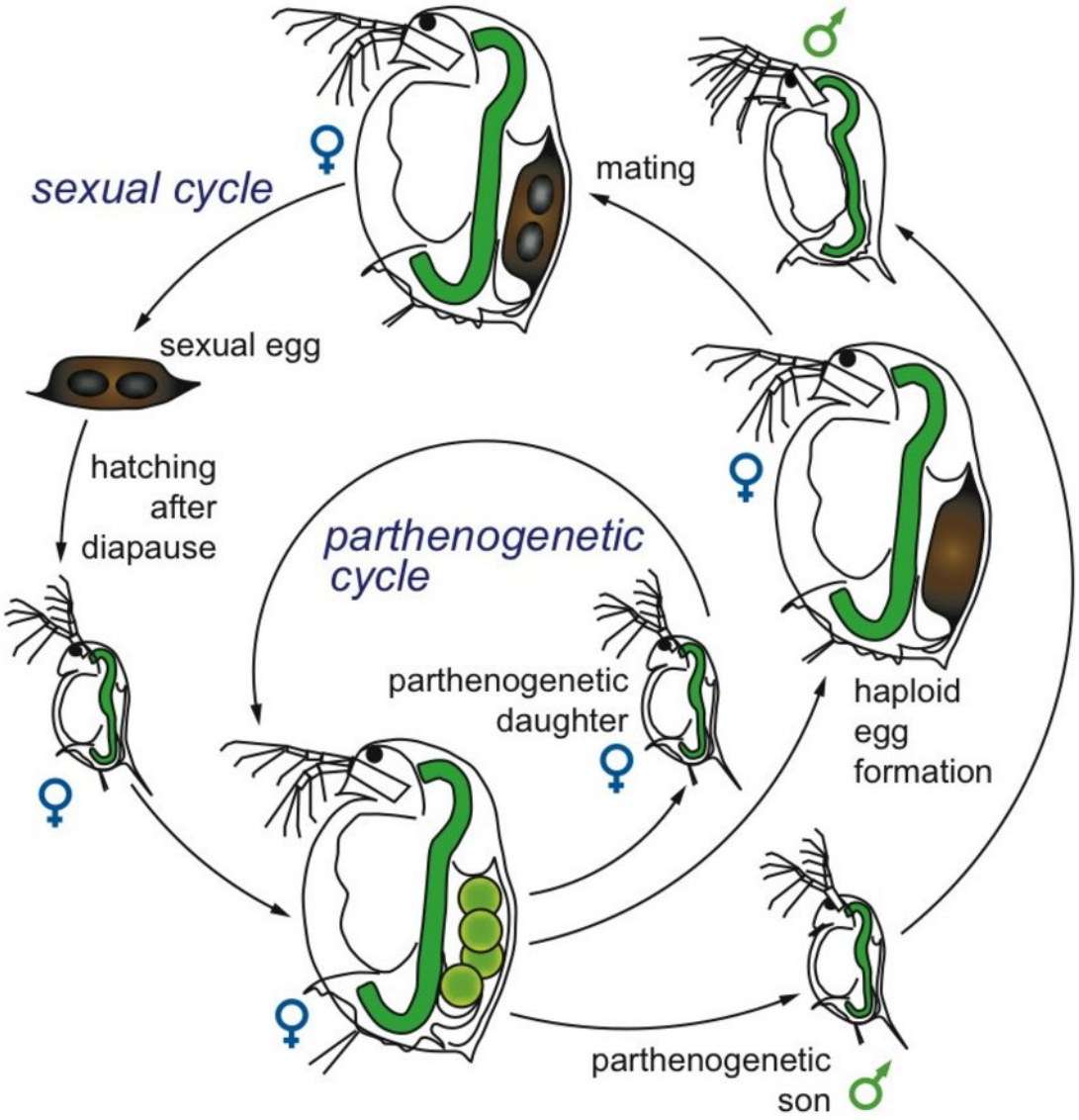

Figure 2.9 Life cycle of a cyclic parthenogenetic *Daphnia*. This diagram depicts the sexual and the asexual (parthenogenetic) life cycle of a *Daphnia*. During the parthenogenetic cycle, females produce diploid eggs that develop directly into daughters. The same female may produce diploid asexual eggs that develop into sons. Male production is under environmental control. Furthermore, the same female may produce haploid eggs that require fertilization by males. These eggs are then enclosed in a protective shell (ephippia) and need to undergo a diapause before female offspring will hatch from them. Drawing by Dita B. Vizoso, Fribourg University.

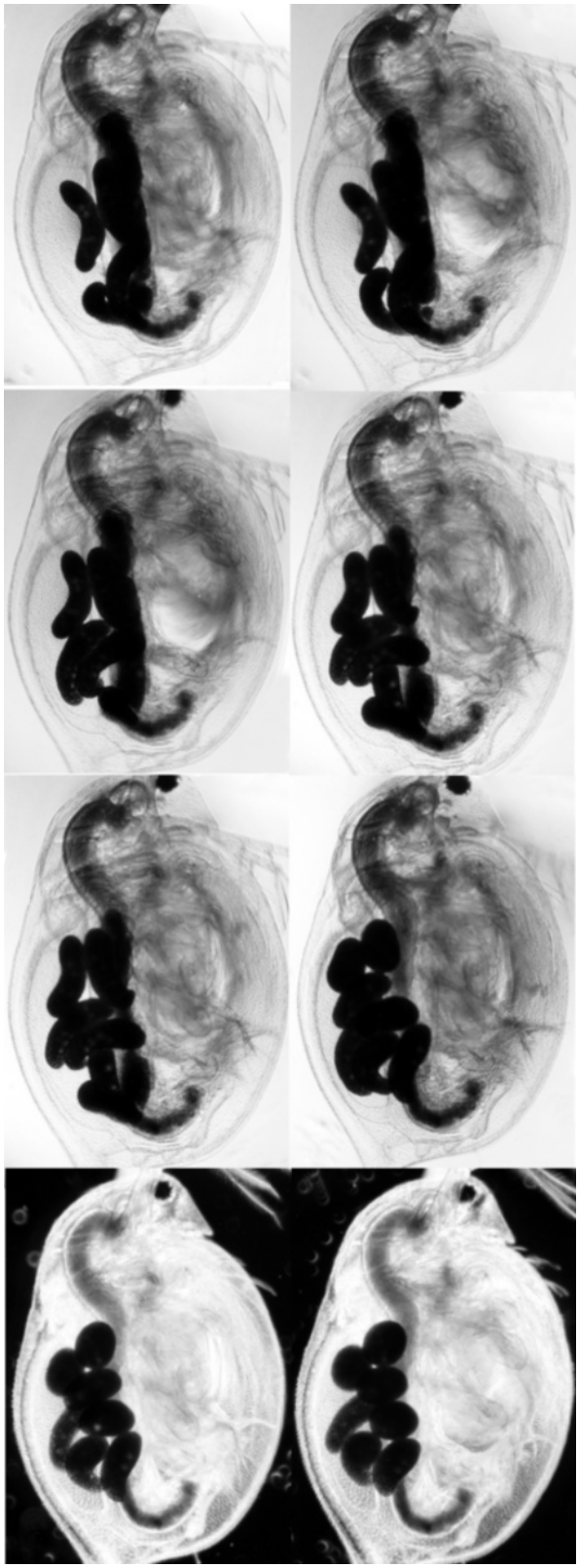

Figure 2.10 Egg laying by *Daphnia magna*. This series of pictures, taken within a 15-minute time span, shows the process of placing eggs into the brood chamber. The eggs are initially sausage shaped but quickly resume a nearly spherical shape. The ovaries shrink during the process of egg laying. In the top pictures, they are clearly visible as a *dark, thick line* parallel to the intestine.

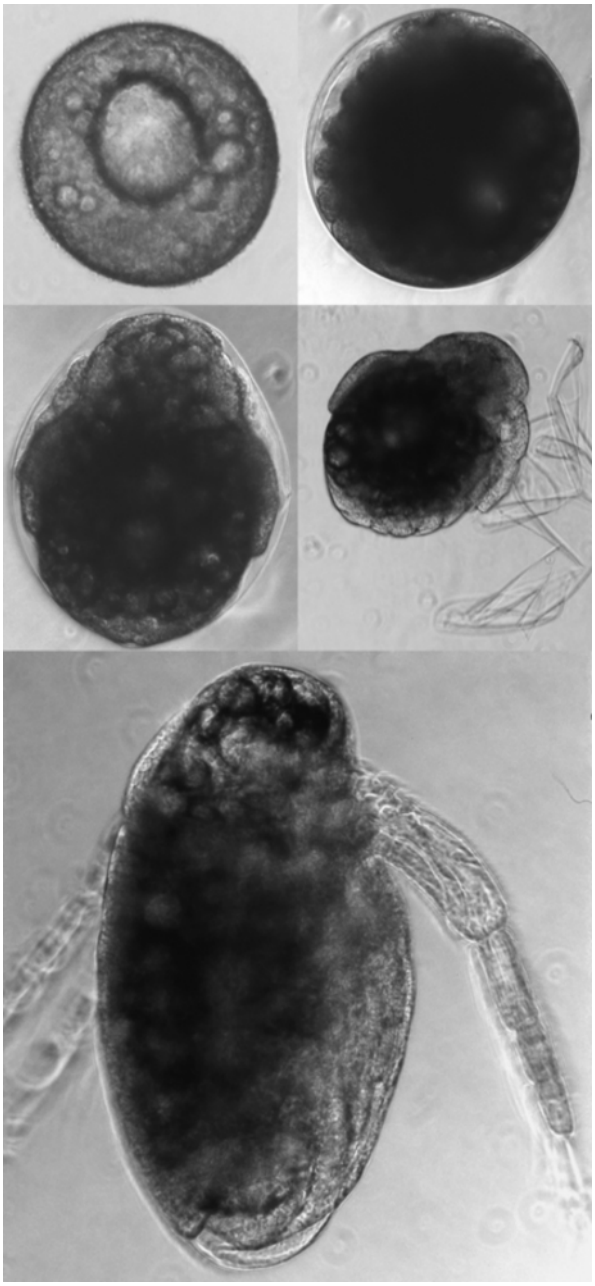

Figure 2.11 Development of *Daphnia* embryos. This sequence shows the development of parthenogenetic eggs and embryos raised in a culture dish. The *top left* picture shows an egg within the first 8 hours after egg laying. The *last* picture shows an embryo of about 2 days of age (at 20°C). Note the egg shell besides the embryo in the *second row, right* picture.

Figure 2.12 Newborn *Daphnia magna*. A parthenogenetic offspring of *D. magna* within the first 12 hours after release from the brood chamber.

ern parts of America and Eurasia and are an excellent example of geographic parthenogenesis (best described for *D. pulex*), may produce diploid resting eggs asexually as well. In the more common sexual *Daphnia* (with cyclic parthenogenesis), resting egg production follows the asexual production of diploid males, which are needed to fertilize the haploid eggs (Figures 2.15 and 2.16). A sexual clone can produce males and females and is capable of self-fertilization. For fertilization, males copulate with females (sometimes two males can be observed on the same female). Fertilization is internal and happens between molting and deposition of eggs into the ephippium. Sperm are either tailless (Figure 2.17) or may contain short, pseudopodia-like extensions. The induction of sexuality seems to be triggered by a complex set of stimuli, the most important possibly being those that go hand-in-hand with a high *Daphnia* density, e.g., increased competition and reduced food availability. Abiotic factors alone, such as decreased day length and lowered temperature, also seem to play a minor role. *Daphnia* in intermittent populations (such as ponds that are likely to dry up during part of the year) have a much higher tendency to produce resting eggs than *Daphnia* in permanent populations, e.g., in large lakes.

The ephippia from the females are released with the shed carapace during molting and sink to the bottom or float with the help of small gas chambers. They may disperse with the wind or with animals (e.g., attached to the feathers of waterfowl) or may drift with the water. Floating ephippia may be driven by winds to form large piles of resting

Figure 2.13 ***Daphnia magna*** **carrying a resting egg.** The ephippium is easily recognized by its dark color. It usually contains two eggs, which are haploid and require fertilization. In the central part of the body of this female, the filled, greenish ovaries can be recognized, which apparently contain several eggs for the next (parthenogenetic) clutch. This female was isolated from a rock pool population in southern Finland. Note that there are some epibiontic peritrich ciliates attached to the body.

Figure 2.14 ***Daphnia longispina*** **carrying a resting egg.** The ephippium is not yet fully developed. Its wall is still transparent, and the two eggs are visible. These eggs are haploid and require fertilization. This female was isolated from a rock pool population in southern Finland.

eggs in wind-protected parts of ponds and lakes. Depending on the habitat, resting eggs may endure unfavorable seasons (e.g., winter colds, periods of low bio-productivity, summer droughts), and hatching is induced by external stimuli, such as an appropriate photoperiod, light, rising temperatures, or simply the presence of water in a previously dry pond. From resting eggs, only females hatch, which usually produce parthenogenetic eggs themselves but may directly produce resting stages under conditions of a very short growing season.

2.4 Habitat

Daphnia populations can be found in a range of water bodies, from huge lakes down to very small temporary pools, such as rock pools (Figures 2.18 and 2.19) and vernal pools (seasonally flooded depressions). Often they are the dominant zooplanktor and form, as such, an essential part of the food web in lakes and ponds. In many lakes, *Daphnia* are the predominant food for planktivorous fish, at least at times. As a consequence, the *Daphnia* species distribution and life history are closely linked with the occurrence of predators. Typically, *Daphnia* species found in lakes with planktivorous fish are smaller and more transparent than species found in fishless water bodies. Large species such as *D. magna* and *D. pulex* usually cannot survive under intensive fish predation, whereas small species such as *D. galeata*, *D. cucullata*, and *D. hyalina* are usually not found in fishless water bodies. A number of invertebrates are known to prey on *Daphnia*, the best investigated probably being the larvae of the phantom midge *Chaoborus* and the water boatman *Notonecta* and related genera. Although visually-hunting fish usually show a preference for larger prey items, invertebrates may prefer smaller prey or even a specific size class. These differences in size-specific mortality rates are believed to be a key factor in the evolution of *Daphnia* body size.

Figure 2.15 Male *Daphnia magna*. A male *D. magna* from a laboratory population of a clone isolated from a rock pool in southern Finland. Photo by Dita B. Vizoso, Fribourg University.

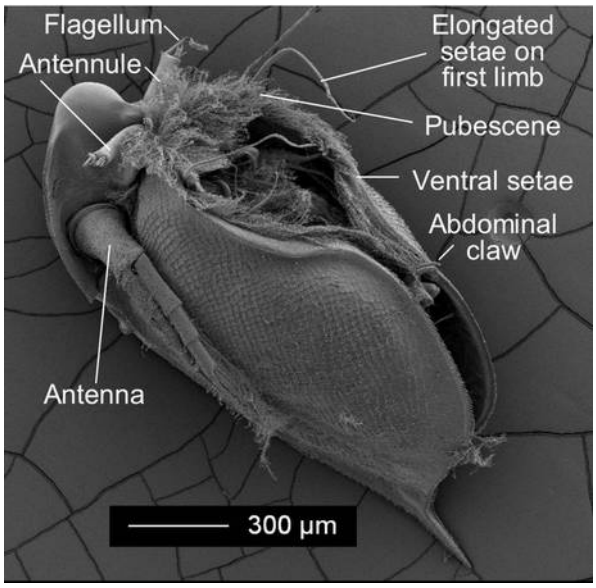

Figure 2.16 Scanning electron microscopic of an adult male *Daphnia magna*. A male *D. magna* from a laboratory population of a clone isolated from a rock pool in southern Finland. Photo by Frida Ben-Ami, Basel University.

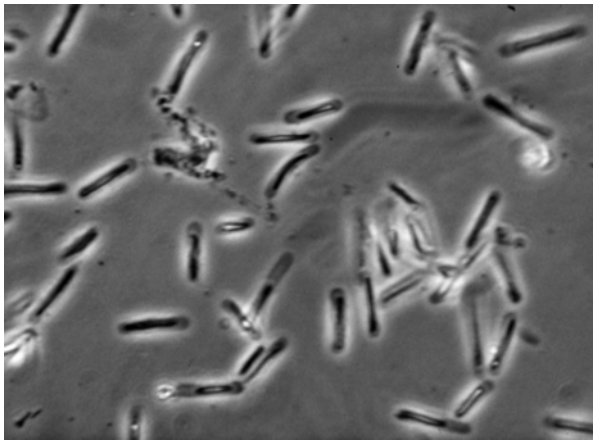

Figure 2.17 Sperm of *Daphnia magna*. Sperm in *D. magna* are tailless, rod-shaped, and up to 9 μm in length. Other *Daphnia* species have sperm with short pseudopodia (not shown here). Sperm is haploid.

High juvenile mortality caused by *Chaoborus* has been suggested as the cause for evolution of larger sizes at birth and to phenotypically plastic adjustments of birth size and growth rates. In contrast, predation by fish has led to smaller sizes and earlier maturation age.

It has been proposed that in water bodies without predation, the composition of *Daphnia* species is influenced by size-dependent competition, with larger species out-competing smaller species. There is, however, some debate about the efficiency of size-dependent processes in the absence of predation.

The water quality of *Daphnia* habitats can vary widely. A pH between 6.5 and 9.5 is acceptable for most species, with the optimum being between 7.2 and 8.5. Salinity should usually be below 5% of seawater (about 1.5 grams of sea salt per liter), but some species can tolerate much higher salinities, such as. *D. magna,* which can be found in up to 20% seawater.

2.5 Behavioral Ecology

The English name for *Daphnia*, waterflea, originates from the jumping-like behavior they exhibit while swimming. This behavior stems from the

Figure 2.18 Rock pools with *Daphnia* populations on four islands in the Tvärminne archipelago in southern Finland. These pools are part of a metapopulation of *D. magna*, *D. pulex*, and *D. longispina*. In the background, the Baltic Sea is visible.

beating of the large antennae, which they use to direct themselves through the water. The rapid downbeat produces a quick upward movement, whereas the relatively high density of the animals creates a sinking. Motionless *Daphnia* sink rapidly to the ground.

A second well-known behavior of *Daphnia* is diel vertical migration, in which they migrate toward upper levels of the water body during nighttime and then back downward during the early morning and daytime. This behavior probably developed as a predator avoidance strategy. During daylight, the *Daphnia* hide from fish that hunt visually by moving to darker depths, whereas during nighttime, they take advantage of the richer food (planktonic algae) in the well-illuminated upper water levels. Inverse diel vertical migration has been described as a strategy to escape other predators that migrate themselves.

Part of the behavioral repertoire of *Daphnia* that is key to diel vertical migration is phototaxis. Clones of *D. magna* vary strongly in their phototactic behavior. Phototactic-positive genotypes spend much time in the upper water level, whereas phototactic-negative genotypes spend most of their time close to the bottom sediments. Phototactic behavior is also influenced by the presence of fish. If *Daphnia* sense that fish are present in the water, they behave more phototactically negative than they would otherwise.

Daphnia are also known to migrate toward or away from the banks of ponds or lakes. Again, the

most likely explanation for this behavior is predator avoidance.

Certain species of *Daphnia*, e.g., *D. magna*, can be observed clinging occasionally to plants or other substrate. Furthermore, they may browse over the surface substrates to pick up small particles. This behavior is more apparent when food is limited and seems to enrich the diet. The stirring movement of the *Daphnia* brings small particles into suspension, which are then ingested by filter feeding.

2.6 Evolutionary Genetics

Following the pioneering work of P.D.N. Hebert, the population genetics of *Daphnia* have been intensively studied around the world. This study was facilitated by allozyme electrophoresis (more recently also microsatellite loci), which revealed a fair degree of enzyme polymorphism in many populations and opened the door for many studies on migration and gene flow (e.g., population divergence, isolation by distance, F-statistics), hybridization (many *Daphnia* species tend to form hybrids), inbreeding (small pools may be colonized by a few clones that subsequently inbreed), and clonal selection (clone frequency changes across the summer season have been frequently observed). It also helped explain phylogenetic relationships among species (later refined with DNA data).

Because of their clonal reproduction, *Daphnia* present a superb tool for quantitative genetic studies, which can enhance our understanding of their evolutionary ecology. Within- and between-clone comparisons can demonstrate genetic variation for various traits within and between populations, thus helping to reconstruct the evolutionary history of a population. For nearly every trait that has been investigated, genetic variation has been reported. Examples include age and size at maturity, size at birth, aging, reaction norms for life history traits, vertical migration, phototactic behavior, fish escape behavior, production of defense spines and helmets, resistance against parasites, immune response, competitive ability, Malthusian growth rate, carrying capacity under stable conditions, and many more. Furthermore, complex matrices of genetic covariances among traits have shown that, in a first approximation, most traits share some covariance with other traits, indicating that an evolutionary change of many traits is constrained by the evolution of other traits.

Figure 2.19 Dry rock pool with *Daphnia magna* populations on an island in the Tvärminne archipelago in southern Finland. The *upper photo* shows a dry pool in July 2003. The *lower photo* shows a close-up of the sediment surface in the pool. The dry sediment has a crust of dead *D. magna*, most of them carrying a resting egg. The *Daphnia* can hatch from these resting eggs as soon as the pool is refilled with rain water.

For those *Daphnia* species that have been karyologically investigated, between 20 and 24 chromosomes have been counted (2N) (Zaffagnini 1987).

With the announcement that the genome sequence of *D. pulex* will be produced in 2005, the genetic study of *Daphnia* is about to enter a new phase. With *Daphnia*, one of the first organisms with a well-known ecology will be sequenced, which is a milestone in the field of ecological genetics. Other molecular tools have been developed in parallel, not only for *D. pulex* but also for *D.*

magna (e.g., microarrays, expressed sequence tags (ESTs)).

2.7 Population Dynamics

Daphnia populations vary strongly in density throughout the growing season. They typically go through pronounced cycles, with densities varying by more than seven orders of magnitude within a single season. A number of studies have refuted the belief that cycles are largely influenced by abiotic conditions; now it is commonly believed that abiotic conditions play a role only in limiting the growing season, not as a factor shaping the population dynamics during the growing season. In many populations, density peaks are observed two or even three times per year, and it is not uncommon for populations to disappear entirely during unfavorable seasons. Although it is difficult to make generalizations about *Daphnia* population dynamics, a few points are apparent.

In most habitats, *Daphnia* have low density or completely disappear during part of the year, usually the cold or the dry season. Recruitment in the following growing season is from resting eggs and/or from surviving females. There is rapid population increase in the early season (exponential growth), with doubling times of a few days (down to 3 days at temperatures of 20°C and above). Growth is eventually slowed down by density-dependent competition, usually because of food shortage; however, predators may contribute as well. During this part of the season, parasites seem to play little role in affecting population numbers. The peak in *Daphnia* density usually follows a peak in algae density and may be followed by the clear-water phase in which the *Daphnia* effectively remove most of the phytoplankton from the water. The resulting food shortage leads to a rapid decline in *Daphnia* density. In large eutrophic lakes in temperate regions, phytoplankton and *Daphnia* may go through two density cycles (a spring and a summer peak), whereas in nutrient-poor lakes, only one peak may occur in mid-season. In small water bodies such as rock pools and vernal pools, the dynamics may look very different, depending on the expected length of the growing season. In pools with a very short growing season (e.g., Arctic and desert pools), *Daphnia* populations may produce resting eggs after only one or two generations, which curtails the exponential growth phase early. In longer-lasting small pools, populations may go through several population cycles within one season. Factors that increase population growth rate (e.g., eutrophication) or that amplify the response to high population density (e.g., sexual reproduction) increase the likelihood of more cycles occurring.

Daphnia parasites are most commonly observed after the first peak in population density. However, it is not clear whether parasites influence the population dynamics of *Daphnia* in natural populations. Parasites are an attractive ecological force for *Daphnia* population regulation because transmission is often density dependent, and they are found in nearly every *Daphnia* population investigated thus far. Experimental epidemiology with various zooplankton parasites has shown that parasites not only suppress host density but also may bring host populations to extinction (Ebert et al. 2000a). Thus, it seems likely that the dynamics of natural *Daphnia* populations are influenced by parasites as well.

Chapter 3

Some Parasites of *Daphnia*

In this chapter, I give a brief introduction to some endoparasite species of *Daphnia*. Three bacteria, one fungus, four microsporidia, and one parasite of unknown taxonomic classification are described with accompanying photographs. I focus on those parasites that are mentioned frequently in this book.

3.1 Introduction

This book is mainly concerned with the ecology, epidemiology, and evolution of parasites. It does not go into detail about the natural history and taxonomy of parasite species. A second book will deal with these aspects. However, because it is useful to have some basic knowledge about the parasites that are frequently mentioned in this book, I give here a brief introduction to them. More details will be found in the upcoming book, which includes chapters on all known *Daphnia* parasites. Table 3.1 gives an overview about all parasites of *Daphnia* mentioned in this book.

The parasites described in this chapter are by no means more important than any other parasites of *Daphnia*, but they are those that happen to be the most studied, partly because they have been found to be at least locally abundant. Parasites of *D. magna* are predominant because parasites of this well-investigated and largest European *Daphnia* species are best known. Most of my own work on parasites has used *D. magna* as a host. Also, parasites for whose entire life cycle can be completed under laboratory conditions were more intensively studied than the numerous species that we do not currently know how to propagate. Despite this bias in representation, however, the species introduced in this chapter give a good impression of the diversity of parasites known to infect the genus *Daphnia*.

We have a good knowledge of the taxonomic position of only a few endoparasites of *Daphnia*. For some species, we do not even know the approximate position, e.g., *Caullerya mesnili*; therefore, I cannot use a strict taxon-based listing of the parasite species. Instead I provide information on other aspects of their biology, which allows us to categorize them into groups so that they can be easily found. When DNA sequence data are available for more species, taxonomic position will be easier to define (Ebert et al. 1996; Refardt et al. 2002).

3.2 Bacteria

Six species of bacteria have been described parasitizing *Daphnia*. Four of them infect the hemolymph, whereas two are intracellular infections of the fat cells and the eggs, respectively. Bacterial infections are generally harmful to their hosts, drastically reducing host reproductive success.

Bacteria have been observed to infect *Daphnia* either as endoparasites or epibionts. However, only the taxonomy for *Pasteuria ramosa* has been worked out and published thus far (Ebert et al. 1996). The

Parasite (Taxon)	Recorded hosts	Infected tissue or site of infection	Transmission
Pasteuria ramosa (Bacteria)	*D. magna, D. pulex, D. longispina*, other Cladocera	Blood, extracellular	Horizontal, from dead host
White Fat Cell Disease (Bacteria)	*D. magna, D. pulex, D. longispina*	Fat body, intracellular	Horizontal, from dead host
Spirobacillus cienkowskii (Bacteria)	Many *Daphnia* species	Blood, extracellular	Horizontal, from dead host
Aphanomyces daphniae (Fungi)	*D. hyalina, D. pulex*	Body cavity, extracellular	Horizontal, from dead host
Metschnikowia bicuspidata (Fungi)	*D. magna, D. pulex, D. longispina*	Body cavity, extracellular	Horizontal, from dead host
Flabelliforma magnivora (Microsporidia)	*D. magna*	Fat body, ovaries, intracellular	Vertical
Octosporea bayeri (Microsporidia)	*D. magna*	Fat body, ovaries, intracellular	Vertical and horizontal, from dead host
Gurleya vavrai (Microsporidia)	*D. pulex, D. longispina*	Carapace, intracellular	?
Glugoides intestinalis (formerly *Pleistophora i.*) (Microsporidia)	*D. magna, D. pulex*	Gut wall, intracellular	Horizontal, from living host
Ordospora colligata (Microsporidia)	*D. magna*	Gut wall, intracellular	Horizontal, from living host
Larssonia obtusa (= *L. daphniae*) (Microsporidia)	*D. magna, D. pulex, D. longispina*	Fat body, intracellular	?
Pansporella perplexa (Amoeba)	*D. magna, D. pulex, D. longispina, D. hyalina, D. obtusa*	Gut wall, extracellular	Horizontal, from living host
Caullerya mesnili (unknown)	*D. pulex, D. longispina, D. magna, D. galeata, D. obtusa, Daphnia hybrids*	Gut wall, intracellular	Horizontal, from living host
Echinuria uncinata (Nematoda)	*D. pulex, D. magna, D. obtusa, other Cladocera*	Body cavity, extracellular	Horizontal, to second host
Cysticercus mirabilis (Cestoda)	*D. magna*	Body cavity, extracellular	Horizontal, to second host (?)

Table 3.1 List of parasites mentioned in this book.

taxonomy of *Spirobacillus cienkowskii* is in preparation (M. Duffy, personal communication). The other species are either described by their typical pathology or are collectively placed into a group with roughly similar characteristics. Most species do not yet have a scientific name.

The recorded bacteria infect either the hemolymph of the host or are intracellular parasites. Infections of the hemolymph of *Daphnia* make the entire host appear milkish-white, brownish, pinkish, or yellowish. These infections can be seen throughout the body and have been found in many *Daphnia* species. Here I introduce two of these species: *P. ramosa* and *S. cienkowskii.*

In contrast, intracellular parasitic bacteria infect either cells of specific host tissues or eggs of the host while they are in the brood pouch. Here I give a short description of a little-known bacterium known by the name of White Fat Cell Disease. Its categorization into two groups of parasitic bacteria is not a taxonomic classification but a functional grouping.

3.2.1 *Pasteuria ramosa* Metchnikoff 1888

P. ramosa is a Gram-positive bacterium belonging to a distinct clade within the family of the *Alicyclobacillaceae* (Ebert et al. 1996; Anderson et al. 1999; Preston et al. 2003). Other endospore-forming bacteria, such as *Bacillus* and *Clostridium*, are closely related to it.

P. ramosa is most frequently found to infect *D. magna*, but it also infects *D. pulex* and *D. longispina*. It shows a high degree of clone specificity within species (Carius et al. 2001). A few other Cladocera have been described as hosts, but it is not clear whether the parasite was indeed *P. ramosa*. *P. ramosa* was recorded in Europe and North America.

P. ramosa infects the hemolymph and is extracellular (Figure 3.1) (Metchnikoff 1888). Infected hosts stop reproduction, grow large, and the body becomes darkish and nontransparent in light. "Squash" preparations reveal large numbers of large, nearly spherical spores (about 5-μm diameter) or grape seed-shaped pre-spores in the hemolymph (Figure 3.2).

This bacterium causes chronic infections. Infected hosts are totally castrated, i.e., they stop reproducing about 5 to 15 days after infection takes

Figure 3.1 *D. magna* with (*right*) and without (*left*) *P. ramosa* infection. The parasite can be seen as a dark cloudy mass filling the entire body. The brood pouch of the infected female is empty, whereas the healthy female carries a clutch of eggs. This photograph was taken with the light shining from below. The infected host is larger than the healthy female, which is typical for *P. ramosa* infections.

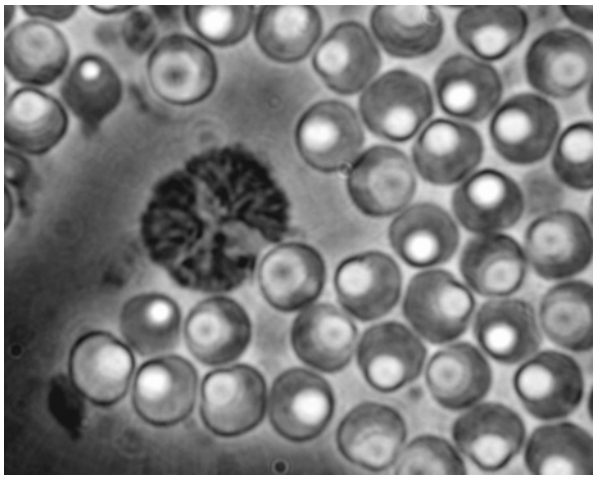

Figure 3.2 Developmental stages of *P. ramosa*. In the final stage of spore development, the host is filled with the round spores that serve as transmission stages. These spores are long-lasting. In hosts in the terminal stage of an infection, one often observes a few cauliflower stages, suggesting that some spores germinate to start another growth cycle. The cauliflower stage is the first stage of *P. ramosa* that is clearly visible after an infection.

place. In contrast to most other *Daphnia* infections, the hosts can live for a long time after the parasite has reduced their fecundity. In the laboratory, death often occurs only 40 to 50 days after infection. At death, hosts are filled with transmission stages (normally around 10 to 20 million spores per host, but up to 80 million spores have been observed). Infected hosts are often larger than uninfected controls. This form of parasite-induced gigantism is believed to be adaptive for the parasite (Ebert et al. 2004).

The development of *Pasteuria* is comparatively slow. At 20°C, 10-12 days after infection of young hosts, the first "cauliflower" stages (sensu Metchnikoff) (Figure 3.2) can be seen. Four days later, alongside the cauliflower type, microcolonies (fractions of these rosettes, with some cell associations consisting of only 2, 3, or 4 cells attached to each other at the pointed end) can be seen. These are branches of the microcolonies, which break away. Each branch eventually forms a single spore that resembles grape seeds. In the grape-seed stage the endospores increase in size until, fully developed, they have a diameter of about 5 μm. These endospores are the transmission stages. They are clearly visible with a light microscope. Details about the ultrastructure of *P. ramosa* can be found in Ebert et al. (1996).

Transmission is strictly horizontal (waterborne) through spores released from the remains of dead, formerly infected hosts. No vertical transmission has been observed. Mud samples from ponds with infected populations are infectious, indicating the role of pond sediments as a parasite spore bank. Samples from sediment cores can be infectious after several decades (Decaestecker et al. 2003). Experimental transmission was possible at 15°C, 20°C, and 25°C without any noticeable difference (Ebert et al. 1996). Transmission stages are released only after the death of infected hosts. Spores liberated from the host cadaver come in contact with uninfected *Daphnia* and cause infections. Thus, *P. ramosa* follows a sit-and-wait strategy. It is not clear whether infection results from ingestion of spores or whether the parasite penetrates the epidermis of the host. The latter has been shown to be the mechanism of infection of *P. penetrans* (note the name!) infecting soil nematodes. In the laboratory, infections can be produced by grinding up infected hosts and adding the resulting spore suspension to host cultures.

Figure 3.3 ***D. magna* with (*left*) and without (*right*) *S. cienkowskii* infection**. The red color of the infected host is the best indicator of the bacterium. The females were collected from a natural rock pool population in southern Finland.

3.2.2 *Spirobacillus cienkowskii* Metchnikoff 1889

This bacterium has been recorded from a wide range of species including *D. magna*, *D. pulex*, *D. longispina*, *D. hyaline*, *D. obtusa*, *D. ambigua*, *D. curvirostris*, *D. laevis*, *D. dentifera*, and several genera of other Cladocera including Sida, Simocephalus, Chydorus, and Ceriodaphnia. The species has been described from sites in Europe, Africa, and North America.

This bacterium infects the hemolymph of its host. The entire host becomes pinkish-red (Figure 3.3). Hosts with well-developed infections can be easily recognized by the bright scarlet red color of their hemolymph (Figure 3.3). This color is caused by carotenoids (Green 1959) and is much more opaque than the color of hemoglobin in the blood, which is sometimes seen in *Daphnia* from habitats with low oxygen (compare Figure 2.7). During early stages of infection, infected animals are more whitish-pale and resemble hosts infected by other blood parasites. The bacterium itself is hardly visible with standard light microscopy.

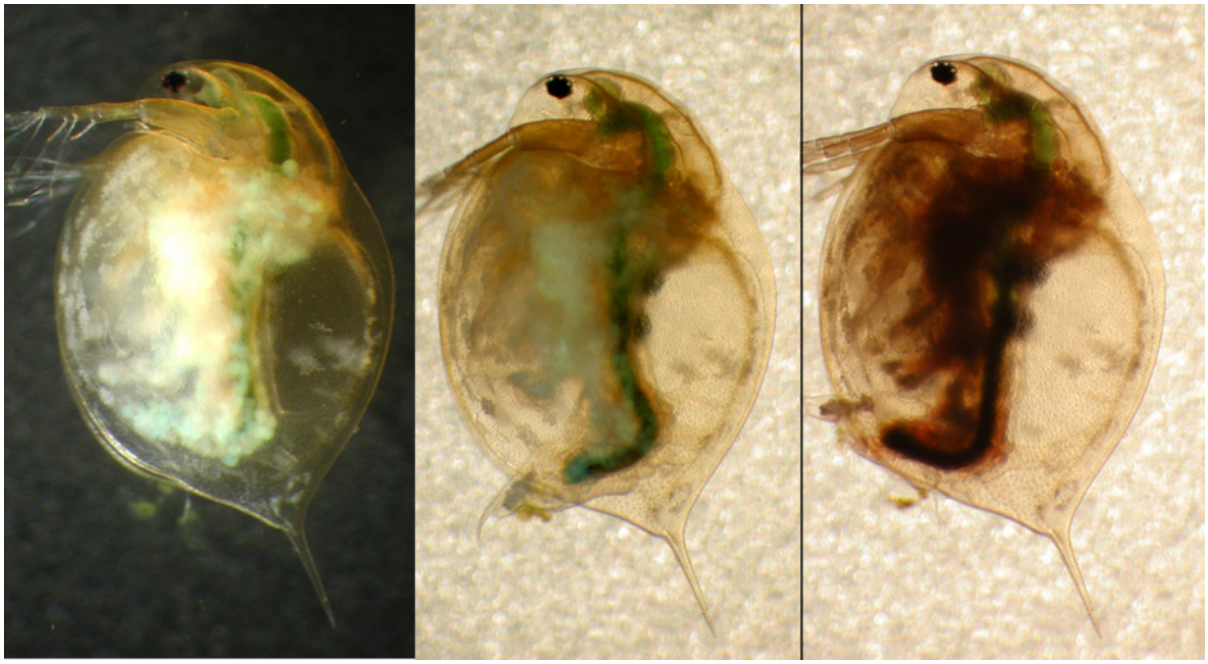

Figure 3.4 ***D. magna* with WFCD**. The same animal is shown under three different light conditions, with light coming from the top (*left*), from the bottom (*right*), and from the top and bottom (*center*). Note that the infected fat cells become less visible with light shining through the animal.

Figure 3.5 WFCD in *D. longispina*. *D. longispina* from a natural rock pool population in southern Finland.

Metchnikoff (1889) described the length of the life cycle of the bacterium as about 5 days. The life cycle includes several morphological forms, including ovals, rods, spirillae, filaments, and round spores. Hosts collected from natural populations in the terminal stage (red color stage) survive only 1-3 days under laboratory conditions and usually carry no eggs (Duffy et al. 2005).

Transmission is strictly horizontal. Prevalence can reach 10 to 15% for short time periods (Duffy et al. 2005).

3.2.3 White Fat Cell Disease

WFCD is caused by a small coccoid pathogen, most likely a bacterium. Infections with this bacterium have been recorded in *D. magna, D. pulex,* and *D. longispina*. Clones of *D. magna* have been found to differ in their susceptibility to WFCD (Decaestecker et al. 2003). The disease has been found only in Western and Northern Europe thus far.

The causative agent of WFCD is hardly visible with light microscopy. Infected hosts have bright white fat cells with a slight greenish shine that

is visible only in reflected light (Figures 3.4 and 3.5). The infection does not show the fuzzy spread through the body cavity that is seen with other parasites infecting the fat cells and ovaries (e.g., *Octosporea bayeri*). Usually, the infected tissue is clearly distinguishable from other tissues.

WFCD is rather harmful. It usually kills the host within 2 weeks, often much more quickly. Less virulent infections have been observed as well. Fecundity drops strongly with disease progression, and infected hosts have stunted growth.

Transmission is strictly horizontal. Transmission stages are released from dead hosts. There seems to be no transmission from living infected hosts and no vertical transmission.

3.3 Fungi

Several species of fungi have been observed parasitizing *Daphnia* and other Cladocera. Taxonomically, they are poorly understood. They vary strongly in their appearance and their effects on their hosts. Fungal infections are generally harmful to their hosts, drastically reducing host reproductive success and survival.

Some species may not be obligate parasites, opening the possibility to culture them on an artificial medium (Couch 1935; Prowse 1954; Whisler 1960). Indeed, it has been reported that the endoparasites *Aphanomyces daphniae*, *Metschnikowia bicuspidata*, and the epibiontic *Amoebidium parasiticum* can be cultured *in vitro*, which opens up tremendous possibilities for experiments. To my knowledge, no other parasite group can currently be cultured outside *Daphnia*.

Host specificity seems to be rather low in fungi infecting crustaceans. From my experience, the parasitic fungi of *Daphnia* are the most difficult to work with and to identify. On the other hand, parasitic fungi seem to be the most devastating diseases of *Daphnia*, often killing the hosts quickly or destroying the broods.

3.3.1 *Metschnikowia bicuspidata* (Metschnikov) Kamenski

This yeast is better known by the names *Monospora bicuspidata* and *Metschnikowiella bicuspidata*. It has been recorded from *D. magna*, *D. pulex*, and *D. longispina* as well as from a number of other crustaceans. It appears, however, that under this name a complex of similar species has been described.

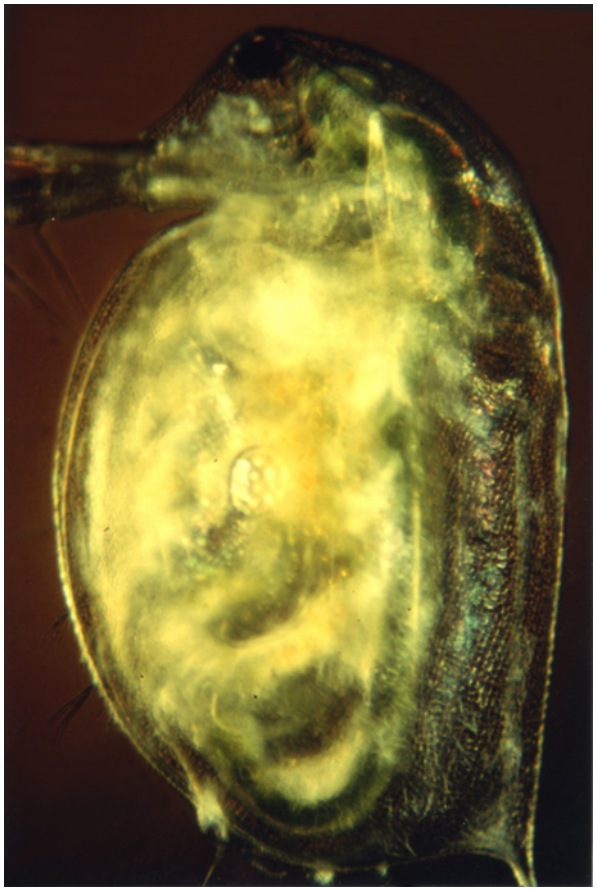

Figure 3.6 ***D. magna* with an infection of *M. bicuspidata*.** This female was infected with a suspension of spores. The host is in the terminal stage of infection. The needle-like ascospores of *M. bicuspidata* fill the entire body cavity of the host.

M. bicuspidata is an endoparasitic Ascomycete (Endomycetales). It produces needle-like ascospores, which penetrate the gut walls of its hosts and germinate in the hemolymph (Green 1974). Needle-like spores are usually up to 45 μm long, although they can be up to 90 μm long (Green 1974; Codreanu and Codreanu-Balcescu 1981), and are visible through the transparent body of the hosts (Figure 3.6). The fungus grows inside the host until the entire cavity is filled with the needle-like spores (Figure 3.7). Spores are found in every part of the body cavity, even in the antennae. Hosts in

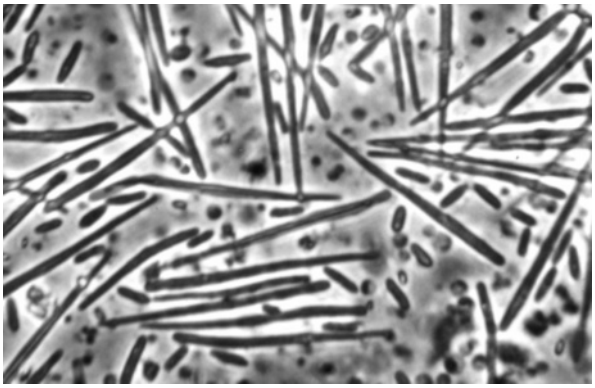

Figure 3.7 Spores of *M. bicuspidata*. These needle-like ascospores of *M. bicuspidata* fill the entire body cavity of the host.

late stages of infections become opaquely white and look as if their bodies are filled with straw.

Successful *M. bicuspidata* infections kill the host within 2 to 3 weeks, sometimes earlier. Host fecundity is reduced, with this reduction becoming stronger as the infection develops (Ebert et al. 2000a, 2000b).

The fungus is transmitted only horizontally (Ebert et al. 2000a). The waterborne spores are ingested with the food and penetrate the gut wall (Metchnikoff 1884). Spores are only released from dead hosts. Grinding up dead hosts in water and adding this suspension to clean cultures allows efficient Transmission of the host (Ebert et al. 2000a).

M. bicuspidata produces local epidemics in *Daphnia* populations, reaching prevalences above 10%. Across a 1-year field study in three English ponds, the average prevalences in *D. magna, D. pulex*, and *D. longispina* were 1.8, 3.0, and 3.7%, respectively (Stirnadel and Ebert 1997). Interestingly, while one pond showed *D. magna* as the most heavily infected host, in another pond close by, *D. pulex* and *D. longispina* were much more predominately infected than *D. magna*, suggesting some degree of local differentiation of hosts and/or parasite.

3.4 Microsporidia

Microsporidia are obligate intracellular parasites. As a group they are clearly distinguished from other eukaryotes, but their taxonomic position is still debated. In older phylogenetic trees, they are often shown to be at the root of the eukaryotes; however, the finding that they possessed mitochondria in their evolutionary past provoked a reconsideration of their taxonomic classification. Now it seems likely that they are a sister taxon to the fungi.

The Microsporidia are the largest group of parasites of *Daphnia*. They are easy to recognize once spores are formed. At 20°C, this takes about 3 to 12 days after infection (Ebert, personal observation). Spores of most species are only a few μm in length (2.5 to 16 μm in the known *Daphnia* parasites) and are usually rather uniform in size and shape. Microsporidians are usually found to be tissue specific (ovaries, fat cells, hypodermis, gut, and epithelium), and the infected tissue can give important clues on the species. Depending on the infected tissue, infections may be clearly visible from the outside (even without a microscope) or are seen only once the host is dissected (e.g., infections of the gut epithelium). Important traits for identification are the number of spores produced by each sporophorous vesicle, as well as the size and shape of the spores. Larsson (1981, 1988, 1999) gives excellent introductions to microsporidia identification. Note that spore size may vary according to culture conditions (e.g., smaller spores were observed at lower temperatures (Friedrich et al. 1996).

Although microsporidian parasites are highly variable in their mode of transmission, a few generalizations are possible. Gut infections are usually transmitted horizontally from the living host. Infections of ovaries are often vertically transmitted. Microsporidian parasites appear generally to be the most host-specific group of *Daphnia* parasites

A number of microsporidian parasites have been found to infect the gut cells of their hosts. These species are difficult to distinguish. Typically, they produce small spores (mostly less than 3 μm long), often in conspicuous sporophorous vesicles that are most easily seen when the gut is dissected. Sometimes only a few sporophorous vesicles are found in the entire gut, but in other cases the entire gut is densely infected. Infections may be localized, often in the posterior part of the gut, so that they are not visible without dissecting the host. Transmission of gut microsporidians is typically horizontal, with spores being released with the host feces and ingested by filter-feeding hosts. All species studied thus far were rather avirulent to their hosts.

The fact that they are highly transmissable, difficult to see, and that they cause little harm to cultures explains the frequent observation that clones that have been kept in laboratories for many years or even decades often carry a microsporidian gut parasite (D. Ebert, personal observation). There must be a large number of publications on *Daphnia* biology that, without the knowledge of the authors, report on experiments with infected animals.

3.4.1 *Flabelliforma magnivora* Larsson et al. 1998

This microsporidium is known only in *D. magna* in Western Europe. The primary site of infection is the adipose tissue, but infection has also been observed in the hypodermic cells and the ovaries (Figure 3.8). Infected hosts are easily recognized by the large spore masses visible in the central part of the body. Spores measure about 2.4 x 4.5 μm and are lightly pyriform, with both poles blunt, often with one surface slightly convex (Figure 3.9) (Larsson et al. 1998).

Infected hosts suffer to some degree from reduced fecundity and reduced longevity (Ebert et al. 2000a). Virulence is, however, comparatively low. Infected hosts may live more than 50 days, and fecundity reduction is between 30% and 50% compared with uninfected controls.

In the laboratory, the parasites are transmitted with nearly 100% fidelity from mother to offspring. It is likely that there is also horizontal transmission, but all attempts for horizontal transmission in the laboratory have failed (Mangin et al. 1995) (Note: In Mangin et al. (1995), *F. magnivora* is named *Tuzetia*.)

An ultrastructural study and description of *F. magnivora* (Microspora: Duboscqiidae) was done by Larsson and coworkers (1998).

3.4.2 *Octosporea bayeri* Jirovec 1936

This parasite was recorded only in *D. magna* (sympatric *D. pulex* and *D. longispina* are not infected) (Ebert et al. 2001) in Europe. It is a parasite of the fat cells and ovaries (Jirovec 1936). In late stages of infections, the host becomes whitish with spores found throughout the body cavity (Figure 3.10). Spores are variable in shape and size but are usually 4 to 5.6 μm in length (Figure 3.11). Larger spores are seen frequently, but these may be abnormally formed. Spores of *O. bayeri* come in two (maybe even three) types (heterosporous), which may have different functions (Vizoso and Ebert 2004; Vizoso et al. 2005).

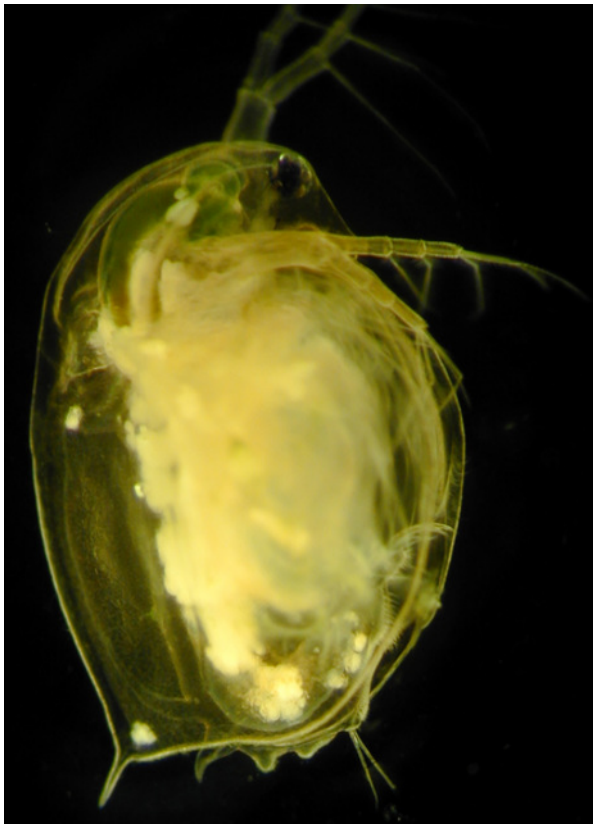

Figure 3.8 ***D. magna*** **with an infection of** ***F. magnivora*****.** This female is in the terminal stage of infection with *F. magnivora*. Hosts infected with this parasite often carry eggs until close to their deaths. The whitish mass is spores.

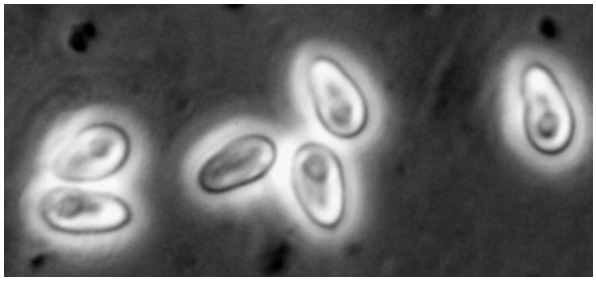

Figure 3.9 Spores of ***F. magnivora*****.**

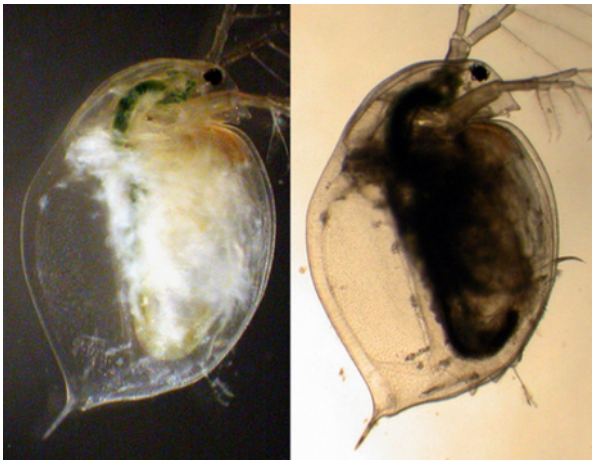

Figure 3.10 ***D. magna*** **with an infection of** ***O. bayeri***. The same animal is shown under two different light conditions, with light coming from the top (*left*) and from the bottom (*right*).

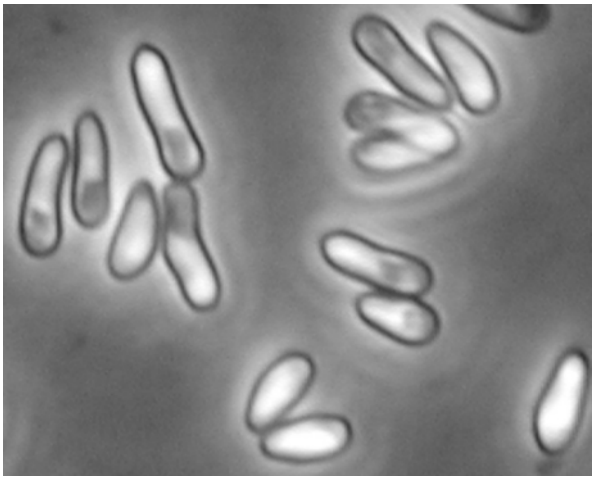

Figure 3.11 Spores of ***O. bayeri***. Note the variability in spore shape and size, which is typical for *O. bayeri*.

Infected hosts have reduced life expectancy and reduced fecundity, with the degree of damage depending on the route of transmission, the host and parasite genotype, and the presence of multiple strains within a host (Vizoso and Ebert 2004, 2005a, 2005b; Vizoso et al. 2005). Fecundity reduction is usually visible only once infections are intense, i.e., after about 15 days.

Transmission is vertical (most likely transovarial) and horizontal. Horizontal transmission occurs only from spores released after the death of the host. Vertical transmission is complete to parthenogenetic eggs but slightly less than 100% to ephippia eggs (Vizoso et al. 2005). The complex life cycle of *O. bayeri* and its interaction with the host life cycle are shown in Figure 3.12. Infections of *O. bayeri* can be cured with a chemical drug (Zbinden et al. 2005), which allows one to obtain uninfected offspring from infected mothers.

In rock pool populations of *D. magna* in southern Finland, this parasite often reaches prevalences of 100%. Early in the season, however, prevalence is usually lower (S. Lass & D. Ebert, manuscript in preparation).

3.4.3 *Glugoides intestinalis* (Chatton 1907) Larsson et al. 1996

This gut parasite was formerly known as *Pleistophora intestinalis* (Larsson et al. 1996). It has been recorded in *D. magna* and *D. pulex* from Western Europe.

Infections with *G. intestinalis* are nearly invisible without dissecting the host. The spores are best seen in dissected guts, where they are recognized by their sporophorous vesicles inside the gut epithelium cells (Figures 3.13 and 3.14). Individual spores are rather small and are oval-to-kidney shaped (about 2.6 x 1.3 μm in 20°C laboratory cultures) (Larsson et al. 1996). There are a number of rather similar species infecting the gut epithelium.

This parasite is rather avirulent, as compared with many other *Daphnia* endoparasites (Ebert et al. 2000a). Infected hosts may live up to 50 days, and fecundity is usually only slightly reduced. External signs of infections are not visible.

Transmission is horizontal from living hosts (Ebert 1995). Spores are shed from the living hosts with the feces and float in the water until the next host ingests them. Vertical transmission does not occur. This parasite is very easily transmitted from host to host. As a consequence, prevalences are often close to 100% among adult animals, and it may be found throughout the year. It is among the few *Daphnia* parasites that may be described as being endemic. The parasite can be kept in even very small cultures of the host, and its presence may escape the attention of the untrained observer.

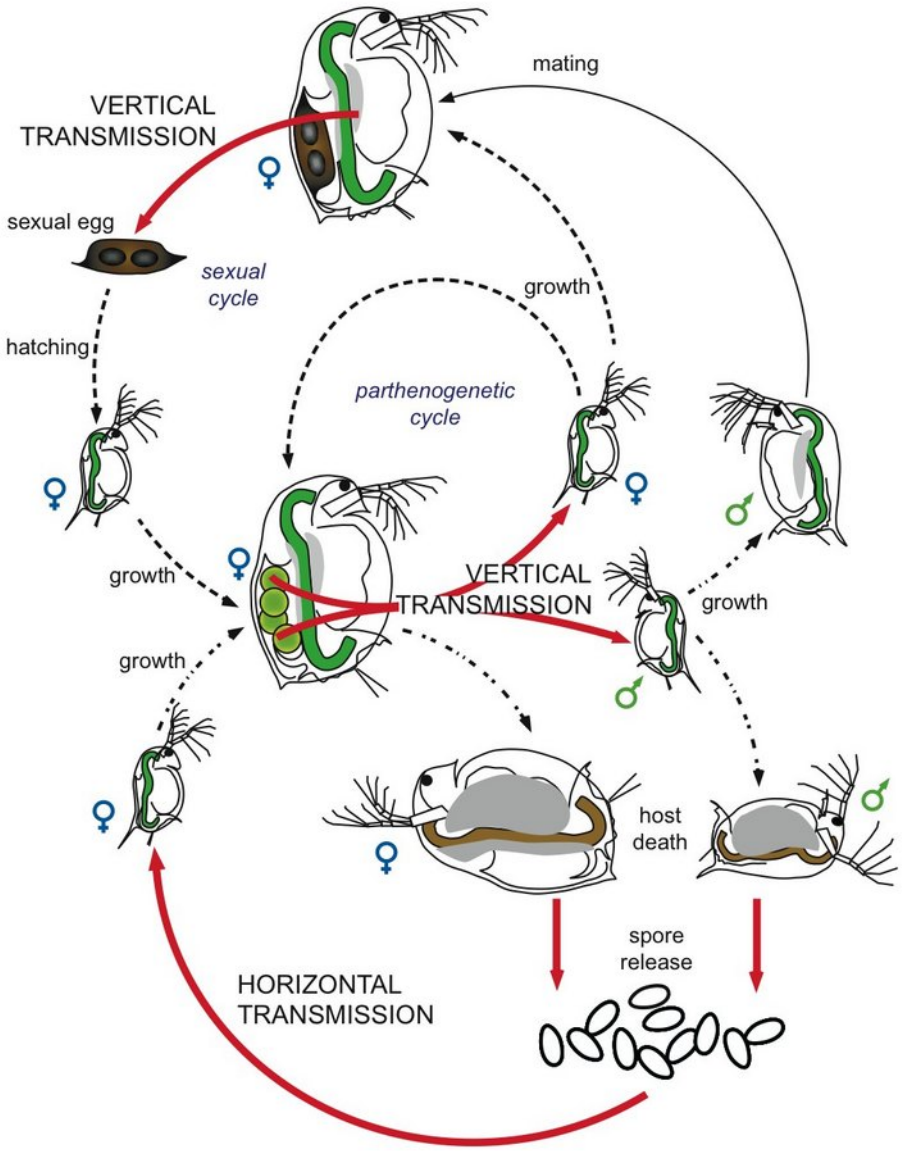

Figure 3.12 Life cycle of *O. bayeri*. Horizontal transmission occurs when infected hosts die and spores are released from the cadaver to the environment. Environmental spores can survive outside the host for several weeks to months (e.g., the entire winter) and can survive the temporary disappearance of their hosts. Infected females can transmit the parasite to their parthenogenetic sons and daughters through vertical (transovarial) transmission. Vertical transmission also occurs in the sexual cycle through the resting eggs. Finally, ephippia may serve as a vehicle for parasite dormancy and dispersal, with a new cycle of vertical and/or horizontal transmission starting after hatching. *Red thick arrows*, transmission of parasite; *black broken arrows*, growth of hosts; *thin black arrow*, interaction between two hosts (Vizoso et al. 2005). Drawing by Dita B. Vizoso.

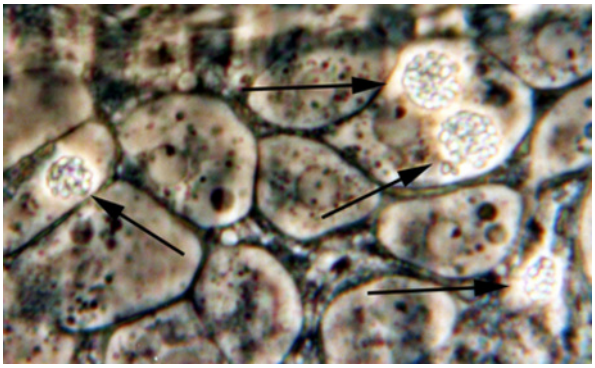

Figure 3.13 Gut cells of *D. magna* with *G. intestinalis*. The *arrows* point to spore clusters of *G. intestinalis* inside cells of the host gut epithelium.

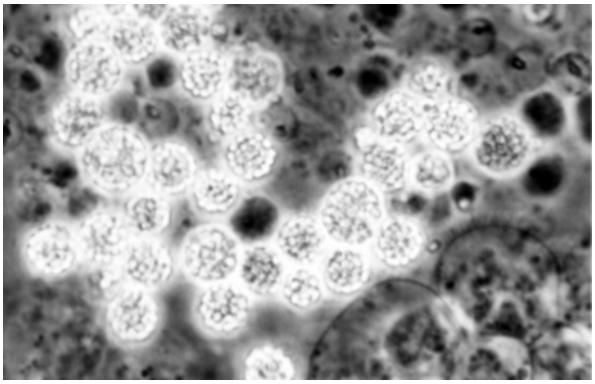

Figure 3.14 Spore clusters of *G. intestinalis*. When infected hosts are dissected, spores and spore clusters are set free.

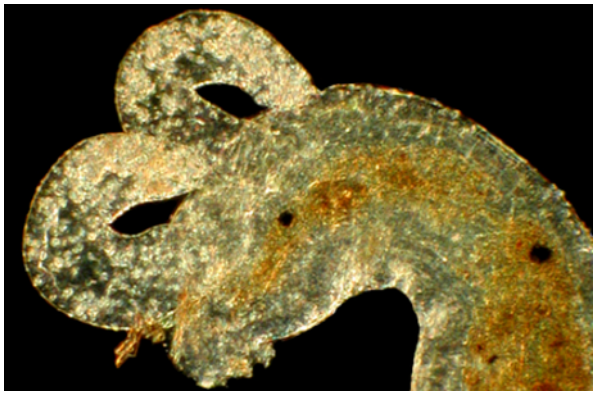

Figure 3.15 Anterior section of the gut of *D. magna* with intense infection of *O. colligata*. The diverticuli are very strongly infected. The light structures are spore masses of the parasite.

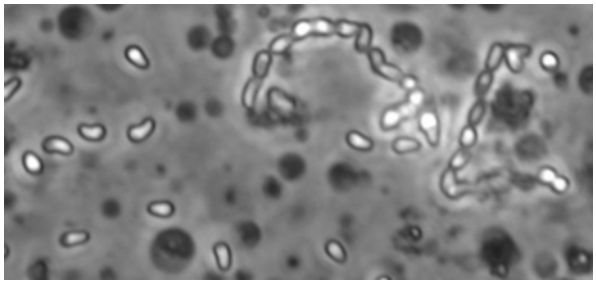

Figure 3.16 Spores of *O. colligata*. Typical for this species is the chain-like arrangement of spores, which can be seen when spores are set free from host cells.

3.4.4 *Ordospora colligata* Larsson et al. 1997

This gut parasite is only known in *D. magna* populations in Western and Northern Europe (Larsson et al. 1997). It is superficially similar to *G. intestinalis* (Chatton 1907) in that it invades the gut epithelium of *D. magna*, where complete development takes place. Infections with *O. colligata* are nearly invisible without dissecting the host (Figure 3.15). The spores are best seen in dissected guts, where spore clusters are seen inside the gut epithelium cells. Individual spores are pyriform and slightly larger (2.9 x 1.5 μm in 20°C laboratory cultures; Figure 3.16) (Larsson et al. 1997) than spores of *G. intestinalis*.

Ordospora colligata is rather avirulent, as compared with many other endoparasites of *Daphnia* (Ebert et al. 2000a). Infected hosts may live up to 50 days, and fecundity is usually only slightly reduced. External signs of infections are not visible.

Transmission is horizontal (Ebert et al. 2000a). Spores are shed from the living hosts with the feces and float in the water until the next host ingests them. Vertical transmission does not occur. This parasite is very easily transmitted from host to host. As a consequence, prevalences are often close to 100% of all adult animals. The parasite can be kept in even very small cultures of the host, and its presence may escape the attention of the untrained observer.

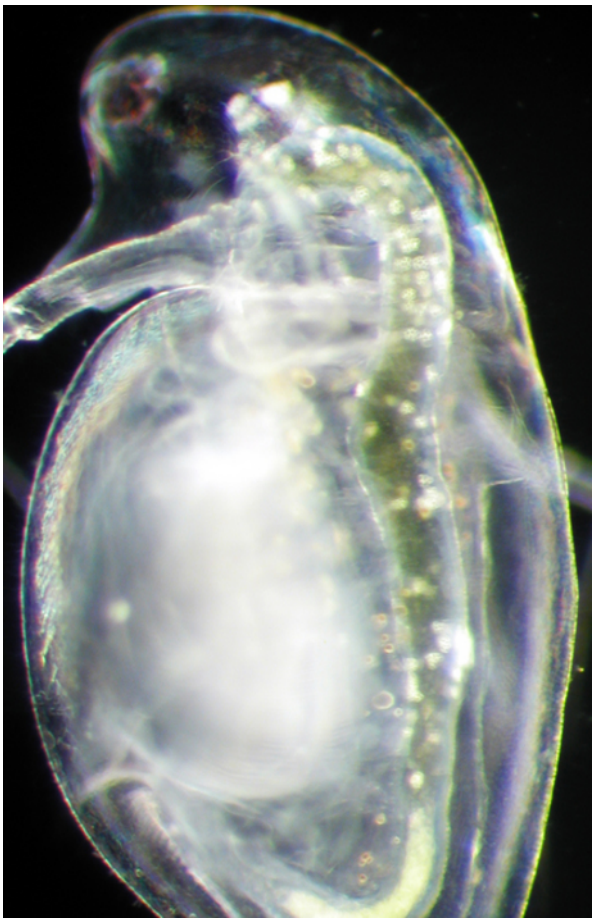

Figure 3.17 *D. galeata* infected with *C. mesnili*. Clusters of *C. mesnili* can be seen on the gut as white, roundish spots.

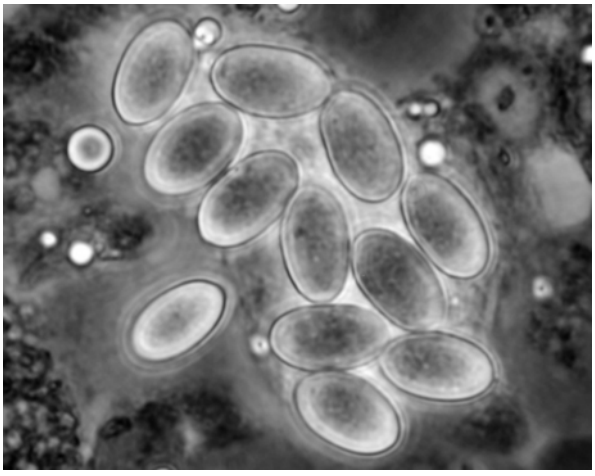

Figure 3.18 Spore cluster of *C. mesnili*.

3.5 Unknown Classification

3.5.1 *Caullerya mesnili* Chatton 1907

This parasite has been recorded in several *Daphnia* species throughout Europe: *D. pulex*, *D. longispina*, *D. magna*, *D. galeata*, *D. obtusa*, and *D. galeata x hyalina* hybrids. It is easily identified by its large spore clusters (up to 100 μm in diameter) consisting of 8-20 oval-shaped spores 10-16 x 8-12 μm (Chatton 1907). The clusters are found inside the gut epithelium, not in the body cavity (Figures 3.17 and 3.18). Infections have not been seen in the gonads.

Bittner et al. (2002) described this parasite as rather virulent. Laboratory-infected hosts have hardly any eggs, and survival is strongly reduced (fewer than 20 days on average). The parasite may drive experimental populations of *D. galeata* to extinction (Bittner et al. 2002). It also strongly influences competition among *Daphnia* species.

Transmission is horizontal from living hosts. Transmission stages leave the gut of the host and are ingested by other filter-feeding *Daphnia*. No vertical transmission was observed. A large-scale screen for this species in many pre-alpine lakes revealed that it is rather common, reaching prevalences of up to 50%.

The taxonomic position of this parasite remains unclear. *C. mesnili* was classified as a Haplosporidium (Chatton 1907; Green 1974), but this classification is certainly not correct. R. Larsson (personal communication) speculated that it may be related to Coelosporidium, a group of not-yet-classified parasites (see, for example, Lange 1993).

Chapter 4

Parasitism in Natural Populations

In this chapter, I summarize what we know about parasite abundance in natural populations. I review longitudinal and comparative studies on the presence of parasites in *Daphnia* and other Cladocera populations to derive general patterns. Although no strong patterns have emerged thus far, some trends are apparent. In the same habitat, larger host species seem to have more parasites than smaller species. One study also reported more parasite species in older and larger host populations. More parasite species and a higher prevalence of parasites were found in ponds than in lakes. In fishless ponds, parasites seem to be more prevalent in summer and fall, whereas this trend is not found in lakes. A number of studies showed that parasites have strong effects on host fecundity.

4.1 *Daphnia* Microparasites in Natural Populations

A first step in understanding the role of naturally occurring parasites on the biology of their hosts is to assess their distribution in natural populations. Parasite abundance is usually expressed as prevalence (determined in most field studies as the proportion of adult hosts or adult females that are infected). A number of investigations on prevalence patterns of *Daphnia* parasites have been conducted. I will summarize these briefly below, followed by a general discussion. Readers who are less interested in the details of these studies may jump directly to the next section, "Generalizations about Parasitism in Natural Populations".

Field studies on parasitism in *Daphnia* and related Cladocera can be grouped into two categories: longitudinal studies, which conduct time series-based research on samples taken in regular intervals from the same body of water; and comparative studies, which use one or a few samples from many bodies of water. Here studies are discussed separately within these two groups and are presented in chronological order. Only studies based on a large number of samples are included. Tables 4.1 and 4.2 give an overview of all studies discussed here.

4.2 Overview of Epidemiological Field Studies

4.2.1 Longitudinal Studies

Table 4.1 gives on overview over all longitudinal field studies on Cladoceran parasites.

Green (1974) conducted a 4-year longitudinal study of Long Water at Hampton Court, UK that included several species of Cladocerans but no *Daphnia*. It is not known whether this pond contained planktivorous fish. Green observed that some parasite species (also known to infect *Daphnia*) had a seasonal abundance pattern. For instance, *Pasteuria ramosa* and *Spirobacillus cienkowskii* were typ-

Host species	Parasite(s) studied	Duration of study, number of sites	Reference(s)
Various Cladocera, no *Daphnia*	Entire parasite community	4 years, 1 pond	(Green 1974)
D. pulex	Only *Thelohania* sp.	3 years, 1 pond	(Brambilla 1983)
Holopedium gibberum	Only 2 microsporidian species	1 summer season, 1 lake	(Yan & Larsson 1988)
D. pulex	Only *Larssonia daphniae*	3 years, 1 pond	(Vidtmann 1993)
D. obtusa	Only 1 unknown trematode parasite	4 years, 7 ponds	(Schwartz & Cameron 1993)
D. magna, *D. pulex*, *D. longispina*	Entire community: 17 parasite and epibiont species	1 year, 3 ponds	(Stirnadel & Ebert 1997)
D. galeata, *D. hyalina*	Entire community: 8 endoparasites	3 years, large lake	(Bittner 2001)
D. magna (and others)	Entire community: 8 endoparasites and 6 epibionts	2 summer seasons, 2 ponds	(Decaestecker 2002) (Decaestecker et al. 2005)
D. galeata x *hyalina* species complex	Only *Caullerya mesnili*	Irregular sampling over several seasons	(Wolinska et al. 2004)
D. dentifera	Only *Spirobacillus cienkowskii*	1 season, 5 lakes	(Duffy et al. 2005)
D. magna	Only *Pasteuria ramosa*	1 season, 1 pond	(Mitchell et al. 2004)

Table 4.1 Longitudinal studies of parasitism in natural Cladoceran populations.

Host species	Parasites	Number of sites	Reference
All Cladocera	Entire parasite and epibiont community	67 rock pool populations	(Green 1957)
Various *Daphnia* species	Entire parasite community	43 populations (ponds and lakes)	(Brunner 1996)
D. pulex, *D. longispina*	Entire community, mainly *Larssonia* sp.	50 *D. pulex* and 25 *D. longispina* rock pool populations	(Bengtsson & Ebert 1998)
D. magna	Entire parasite and epibiont community	137 rock pool populations	(Ebert et al. 2001)

Table 4.2 Comparative studies of parasitism in natural Cladoceran populations.

ically found only between April and December. The abundance patterns of other parasite species were not tied to seasons, however, leading Green to conclude that the distribution of certain parasites is influenced by the severity of winter and spring temperatures. He did not discuss the role of host density as an explanatory factor. He did observe, however, that several parasite species have negative effects on host survival and fecundity.

Brambilla (1983) studied the microsporidium *Thelohania* sp. in a *D. pulex* population over a 3-year period in a small, apparently fishless vernal pool in Michigan, USA. He noted that parasite prevalence varied strongly, from 20% to peaks of nearly 100% of adult females. Parasites were present in May and June in all 3 years and were first seen whenever the host density rose above 2-3 animals/liter. One year, however, the parasites disappeared in mid-summer despite high host densities, suggesting that high density alone cannot explain parasite spread. Infection of females with ephippia was never observed. Parasitized animals were usually larger than uninfected hosts but had lower fecundity and survival.

Yan and Larsson (1988) followed the dynamics of two undescribed and very similar microsporidian parasites of *Holopedium gibberum* in a 32-ha Canadian shield lake (maximum depth, 16 m) from April to October 1985. The lake has several planktivorous fish species. Parasites appeared only in July and reached a prevalence of 4%, which they maintained for the rest of the summer. The parasites appeared when host density was high but did not decline when host density decreased. The authors argued that elevated summer temperatures were not the cause of the seasonal occurrence of the parasites. They further rejected the idea that changes in host resistance influenced the abundance pattern of the parasites. They suggest, instead, that the interplay between host and parasite population dynamics may have caused the seasonal changes in prevalence and that predation by planktivorous fish may have further influenced these changes, because infected hosts may be, they speculate, the preferred target of visually hunting fish. Infected hosts had a lower fecundity than healthy hosts and may have had lower survival. In closing, the authors noted that in a survey of 15 other *H. gibberum* populations in shield lakes, 3 additional populations were found to be parasitized by microsporidians.

Vidtmann (1993) studied parasitism by the microsporidium *Larssonia daphniae* (later called *Larssonia obtusa* (Vidtmann and Sokolova 1994)) in a *D. pulex* population in a shallow, fishless, eutrophic pond at the Kaunas Zoological Garden in Lithuania. He observed that although microsporidians were present only during times of high host density, they were nonetheless often absent during periods of high host density as well. Prevalence among adult females peaked in summer at a maximum of 52%, but the average prevalence (all age classes) within seasons and across years was much lower: 0.63% in spring, 3.2% in summer, and 2.4% in fall. Prevalence was generally lower in juveniles and in males. Over 3 years, the microsporidians were seen only from late May to early October. Because this period closely overlaps with the presence of the host, this apparent seasonality may be related to the seasonal occurrence of the host. Nevertheless, Vidtmann (1993) speculated that the delayed onset of *L. daphniae* epidemics in May was a consequence of low spring temperatures.

Schwartz and Cameron (1993) studied an undescribed trematode parasite of *D. obtusa* from seven seasonal, fishless ponds in southeastern Texas, USA over 4 years. They recorded strong within-season, between-year, and between-pond dynamics in the presence of the parasite. Despite recording maximum prevalences up to 79%, they more typically found prevalences to be around a few percents. Large animals were more often infected than small females. Host fecundity was only reduced in infections with three or more parasites per host.

Stirnadel (1994) and Stirnadel and Ebert (1997) studied parasites of *D. magna, D. pulex*, and *D. longispina* in three fishless ponds near Oxford, UK over a period of 1 year (about 10-12 *Daphnia* generations, 65 samples in total). She assessed host density and fecundity together with parasite prevalence, richness, diversity, and host specificity. Overall parasite prevalence (all species combined) was high throughout the year, averaging 84.7% in adult *D. magna*, 53.6% in *D. pulex*, and 38.6% in *D. longispina*. Overall, 31% of *D. magna*, 17% of *D. pulex*, and 11% of *D. longispna* were infected with more than one parasite species. In all three

host species, the fecundity of parasitized females was significantly lower than of uninfected females (>20% reduction in *D. magna*, >25% reduction in *D. pulex*, and >7% reduction in *D. longispina*). Only 2 of the 11 common micro-endoparasites found in these three ponds (17 species in total) showed no specificity within the three *Daphnia* host species; the other nine common parasites infected either only one or two of the three sympatric host species or differed in their host specificity across the three ponds, indicating that the parasites may be specialized for the pond's current or former predominant host community. A few parasite species showed a seasonal pattern (parallel in all three ponds). For example, the microsporidium *Thelohania acuta* and the protozoan *Caullerya mesnili* were never found in winter, whereas other parasites showed no such pattern (Stirnadel 1994).

Bittner (2001; Bittner et al. 2002) investigated the parasites of *D. galeata* and *D. hyalina* in Lake Constance in southern Germany. The lake has a surface area of 538 km^2 and a maximum depth of 252 m and contains several planktivorous fish species. In a 3-year study with regular sampling, eight endoparasites (plus one brood parasite) were found with an average prevalence of 5.6% in *D. galeata* and 15.6% in *D. hyalina*. Five of these eight parasites reached peak prevalences of more than 20% (up to 50%) in at least one host species. Most of the prevalence peaks were found in fall and winter. The most common parasite was *C. mesnili*, which was observed to have a strong negative effect on host fecundity. There was no apparent correlation between host density and parasite prevalence.

Decaestecker (2002; Decaestecker et al. 2005) studied parasitism in *D. magna* over a period of 2 years (April to December each year) in two shallow, eutrophic ponds in Belgium that contain several planktivorous fish species. Eight endoparasite species and six epibiont species were recorded, with microsporidia being the most common group (four species). The overall prevalence of endoparasites was high (95.5% in 1999 and 69.9% in the following year). Severe reductions in fecundity were observed in females infected with *Pasteuria ramosa*, White Fat Cell Disease, *Flabelliforma magnivora*, and *Ordospora colligata*, but hardly any fecundity reduction was found for infections with epibionts. There were no clear seasonal trends in the temporal dynamics, but the sampling period did not cover the entire year. *Daphnia* density was observed to be negatively related with overall endoparasite prevalence, whereas epibiont prevalence correlated positively with *Daphnia* density. Interestingly, parasite species that severely reduced host fecundity did not persist as long in the population and had, on average, lower prevalences than benign species.

Wolinska et al. (2004) studied the parasites of the *D. galeata* x *hyalina* species complex in Lake Greifensee in Switzerland. This lake harbors several planktivorous fish species. The prevalence of *C. mesnili* was as high as 22%, and severe effects on host fecundity were observed. Most interestingly, *D. galeata* x *hyalina* hybrids were frequently infected, whereas *D. galeata* was rarely infected. (The other parental species, *D. hyalina*, was very rare.) The authors speculated that differential parasitism of parental and hybrid taxa may contribute to their coexistence. There was no correlation between host density and parasite prevalence. The authors also reported the occurrence of a bacterial parasite in the haemocoel, which reached a peak prevalence of 7%.

Duffy et al. (2005) studied the dynamics of *Spirobacillus cienkowskii* infecting *D. dentifera* in five lakes during a 5-month period. They recorded a marked prevalence peak (up to 12%) in some of these lakes in fall, which coincided with a drastic drop in the predation rate by bluegill sunfish. An epidemiological model, fitted to the particulars of this system, indicated that the drop in predation rate was enough to account for the occurrence of the *S. cienkowskii* epidemics. Changes in predation pressure cannot, however, explain the strong decline of the epidemics in late fall. The authors speculated that the reduced temperature may cause the termination of the epidemic, but these speculations are not well-supported. Host density as a causative factor for the termination of the epidemic was not discussed.

Mitchell et al. (2004) followed *P. ramosa* infections in a *D. magna* population for a period of 4 months in a small farm pond near the Scottish border in UK. Because their paper concerns the coevolution of this system, little information is given on the epidemiology of the system. *Pasteuria* prevalence increased drastically in mid-August, reached a peak of nearly 30% in late August, and had disappeared by late September.

4.2.2 Comparative Analyses

Table 4.2 gives on overview over all comparative field studies on Cladoceran parasites.

Green (1957) studied parasites and epibionts of Cladocera in 67 rock pool populations in the Skerry islands of southern Finland. These pools were small (3-4 m in length and up to 0.4 m deep) and fishless. Parasite richness declined from *D. magna* to *D. pulex* to *D. longispina*, suggesting that larger *Daphnia* species harbor more parasites. Green found that some parasite species lowered host fecundity more than others, and in one case, he observed that ephippial females were overparasitized. The author suggested that certain species of epibionts compete with each other for space on the host and thus exclude each other at the population level.

Brunner (1996) (D. Bruner and D. Ebert, unpublished observations) investigated single samples from 43 *Daphnia* populations in southern England, mainly west of London. Water bodies ranged from small ponds in parks to large natural ponds and medium-sized drinking-water reservoirs. Most of these ponds were fishless. Ninety-one percent of these populations harbored at least one endoparasitic infection (mainly microsporidians). The average prevalence was rather high. In the more common *Daphnia* species, parasites had an average prevalence of 43% (n = 17) in *D. magna*, 69.7% (n = 17) in *D. pulex*, and 43% (n = 9) in *D. longispina* (all parasite species combined). Among the *D. magna* populations, average prevalence was 58.4% (standard error of the mean (SE), 8.4) in permanent ponds, and only 23% (SE, 6.4) in intermittent ponds. This difference was, however, most likely attributable to the smaller size of the intermittent ponds. As seen in other studies (Stirnadel and Ebert 1997; Decaestecker 2002), the most common parasites of *D. magna* were microsporidian gut parasites.

Bengtsson and Ebert (1998) conducted a similar survey with only one sample per pond in a rock pool metapopulation along the Swedish east coast near Uppsala. In these pools, 24 of 50 (48%) *D. pulex* populations and 9 of 25 (36%) *D. longispina* populations investigated harbored at least one parasite species. Across all ponds, the average microparasite prevalence was 15.5% for *D. pulex* and 9.1% for *D. longispina* (about 30% and 25% when only populations with at least one parasite species are considered). The infections in the pools were primarily attributable to a single, virulent microsporidium species (possibly *Larssonia obtusa* (Vidtmann and Sokolova 1994)), which reduced clutch size by 98%.

Ebert et al. (2001) studied *D. magna* in the same rock pool metapopulation in southern Finland as did Green (1957) (see above and Figure 2.18). Because the ecology of this metapopulation is well known, it was possible to address several aspects of parasite distribution across populations in relation to various pool characteristics. Eight endoparasites and eight epibiont species were found in 137 rock pool populations. The number of endoparasite species per population increased with the age of the *Daphnia* population. Typically, newer populations founded in the year the survey was conducted had no or few parasite species, whereas older populations had increasingly more. Furthermore, large rock pools with presumably larger and more permanent *Daphnia* populations were more likely to harbor parasites than smaller pools. The most prevalent parasite in the Finnish rock pools was the microsporidium *Octosporea bayeri*, which often occurred in a prevalence of 100%. This parasite exclusively infects *D. magna* and was found in nearly 50% of all populations, with much higher percentages in older populations. Surprisingly, Green (1957) found this parasite in only 8.3% of *D. magna* populations.

4.3 Generalizations about Parasitism in Natural Populations

4.3.1 What Can We Learn from Prevalence Estimates?

Prevalence estimates are a common and convenient measure of parasite abundance. They allow the investigator to follow changes in parasite abundance over time and provide a reasonable picture of the degree to which the host population is infested. Prevalence estimates have some limitations that have to be taken into account when doing parasitological research. First, they are usually underestimates, because parasites are only detectable after signs of infection have developed. Infections

by the microsporidium *O. bayeri* are only visible 8-12 days after the infection occurs (Vizoso and Ebert 2004). Infections of *C. mesnili* take 6 days until the parasite is visible (Bittner 2001). Although it is possible to obtain a more accurate measure by keeping the sampled animals for a few days in the laboratory before dissection, this may lead to losses because of mortality before the animals are investigated. Using this method, I found that prevalence estimated in fresh samples might be underestimated by as much as 30% (personal observation). Because most parasites need about 1 week to show the first symptoms, juvenile *Daphnia* usually appear to be uninfected, even if they contracted the disease within the first day of life. Therefore, most investigators studying *Daphnia* parasites concentrate on adult animals.

A second problem regarding prevalence is that it correlates with the expected life span of an infection and therefore, when compared across parasite species, can only provide a rough guideline. The investigator will hardly ever see a parasite that kills its host shortly after it produces the first signs of infection. In contrast, parasites that allow their hosts to stay alive for long periods are observed more often, thus showing higher prevalence. Mathematical modeling has shown that, everything else being equal, the more quickly the parasite kills its host the lower is its prevalence (Anderson 1979).

When comparing reports on parasite prevalence in natural populations, one may want to distinguish between studies that were initiated because the investigator had observed high parasite abundance beforehand and those in which populations were screened at random or for other reasons than to study parasitism *per se*. I know or suspect that the investigations by Green (1974) (but not those in Green 1957, 1964), Stirnadel and Ebert (1997), Brambilla (1983), Yan and Larsson (1988), Vidtmann (1993), and Mitchell et al. (2004) were initiated because rates of parasitism were known to be high. In contrast, this was not the case in the following studies: Brunner (1996), Bengtsson and Ebert (1998), Ebert et al. (2001), and Bittner (2001). Accordingly, the average prevalence estimates in the later studies are mostly lower than in the earlier listed reports. This does, however, show that parasites can be common even in populations that were not specifically chosen because of known high parasite abundance.

4.3.2 Host Body Size and Parasitism

Studies that investigated more than one *Daphnia* species in a given habitat found that within populations, the larger species were more strongly parasitized than smaller species (Green 1957; Stirnadel and Ebert 1997). Because transmission for many parasite species occurs after the host ingests spores with its food (see Chapter 8, Epidemiology, subsection on Transmission), this relationship may be explained by the considerably larger volume of water that the larger *Daphnia* filters. However, alternative explanations, such as differential susceptibility, may contribute to this pattern as well.

4.3.3 Effect of Parasites on Individual Hosts

Several studies looked for the effects of parasites on host fecundity and survival. Because *Daphnia* carry their offspring in their brood chamber for several days before releasing them, clutch size is the most convenient and most often studied trait in relation to infection status. Several studies reported reduced fecundity of infected hosts (Green 1974; Brambilla 1983; Yan and Larsson 1988; Vidtmann 1993; Stirnadel and Ebert 1997; Decaestecker et al. 2005). The degree to which fecundity is reduced varies strongly among parasites, with certain species showing no effect. Interestingly, the number of eggs in a clutch seems to be affected less often than the presence of a clutch in the brood chamber (Bittner 2001; Decaestecker et al. 2005; Ebert et al. 2004; Stirnadel and Ebert 1997). Thus, in many cases it seems that parasites suppress host fecundity totally rather than reducing fecundity to a variable degree. Furthermore, the effect of parasites on host fecundity seems to vary with environmental conditions. For example, Yan and Larsson (1988) found no significant fecundity effect in a large sample of 401 females, whereas in the following year, a smaller sample revealed a strong effect of parasites on host fecundity. Bengtsson and Ebert (1998) found that the degree of fecundity reduction varied across populations.

At least two studies have reported associations (positive and negative) between the production of resting eggs and parasitism. Brambilla (1983) found that ephippial females were never infected, whereas Green (1957) found that ephippial females

were relatively more often infected than parthenogenetic females. The association between gender and parasitism certainly needs further investigation.

The effect of parasites on host survival has been tested in field studies by bringing plankton samples to the laboratory, dividing the individuals into infected and uninfected groups, and then monitoring their survival under controlled conditions. For a number of reasons, I consider this approach to be unsatisfactory. First, infected and apparently uninfected hosts may differ in size, age, and experience. Because parasites often influence growth, it is not possible to correct for these differences easily. Second, the assessment of infection status is often difficult, with strong variation across investigators and among diseases. For example, certain infections may only be recognizable shortly before the death of the host, whereas others can be detected a few days after infection. Thus, comparing infected and uninfected animals from field samples does not allow one to judge the effect of parasitism on host survival in a meaningful way.

4.3.4 Infection Dynamics

All of the longitudinal studies found that prevalence varied dynamically over time, with certain parasite species being seen only over short time intervals. In some cases, the dynamics appear cyclic, with seasonal reoccurrence of parasites (mostly in summer), but for the majority of parasite species, it is unclear what determines abundance patterns. Extreme cases of parasite dynamics have been observed in some of the longer studies, where certain parasites disappeared for extended periods of time and then reemerged without any noticeable reason (Green 1974; Bittner 2001). It is totally unclear whether environmental or evolutionary factors play a role in these extreme dynamics.

A few of the longitudinal studies analyzed the dynamics with respect to host density. Thus far, no study has shown a clear density effect, although density-dependent transmission has been shown in the laboratory (Ebert 1995; Bittner et al. 2002). Some studies observed that parasites first appeared when host density was high, but in contrast to what would be expected if dynamics were driven by density dependence, parasites did not decline when host density declined (Brambilla 1983; Yan and Larsson 1988). This trend has also been observed for parasites of planktonic rotifers (Miracle 1977; Ruttner-Kolisko 1977). Several studies suggested that host density and water temperature are to some degree confounded, because the density of most plankton organisms is high during the warmer periods. Therefore, it is not clear to what degree elevated temperatures play a role in summer epidemics (Green 1974; Brambilla 1983). At least for one microsporidium, it has been suggested that low temperature can hinder transmission (Ebert 1995). There are, however, several reports of parasite occurrence at low (winter) water temperatures, indicating that temperature alone cannot explain the occurrence of epidemics (Stirnadel 1994; Bittner 2001; Decaestecker et al. 2005). A possible explanation could be that parasites do not grow at low temperatures but may be able to persist for some time. The relationship between the spread of parasites in relation to host density and water temperature certainly needs further investigation.

The community-level perspective of Decaestecker et al. (2005) revealed remarkable patterns. *Daphnia* density was observed to be negatively related with overall endoparasite prevalence, whereas epibiont abundance correlated positively with *Daphnia* density. Furthermore, parasite species that severely reduced host fecundity persisted for shorter amounts of time in the population and had, on average, lower prevalences than benign species. The data did not allow a fine resolution of these patterns, but the following interpretation may explain these findings. Higher host density allows parasites to spread and thus increases prevalence. Thus, harmless parasites (such as epibionts) are more abundant when host density is high. Here it is the host that governs parasite dynamics. However, harmful parasites may at the same time reduce the host population growth rate so much that their net effect on the host population is a reduction in density. This reduction in host density destabilizes the parasite population, which leads to short parasite persistence times. Thus, for harmful parasites, the epidemiological feedback between host and parasite governs the parasite dynamics.

The strong dynamics of many parasite species also indicate that studies that use only one or few samples per population to estimate the richness of

the local parasite community are likely to vastly underestimate parasite richness.

4.3.5 Are There Fewer Parasites in Lakes with Fish?

There seems to be a difference in the degree of parasitism in water bodies with and without planktivorous fish. The lower parasite richness and prevalence estimates in lakes with fish predation are underscored by the fact that there are fewer literature reports of *Daphnia* parasites from lakes with fish. Several factors may work together to explain this fact.

First, the likelihood of infection increases with body size (Vidtmann 1993; Stirnadel and Ebert 1997), which is probably a result of both higher filtration rates (and thus higher uptake rates of parasite spores) and an accumulation effect with age. In ponds with high adult mortality, as is typical for populations with planktivorous fish, the average life expectancy of a *Daphnia* is low, and thus, parasites may have a lower chance of completing their development. This reduces not only parasite survival but also parasite transmission, because older infected hosts are those that release most (or even all) of the transmission stages. A prediction of this hypothesis is that parasites found in lakes with high predation pressure should complete their development quickly (short prepatent phase) and thus kill their host early. The most virulent *Daphnia* parasites have been indeed described from habitats with planktivorous fish (Bittner 2001; Wolinska et al. 2004; Duffy et al. 2005). Another prediction is that parasitism rates in lakes should be higher at times when fish predation is low. Indeed, parasitism in Lake Constance is mainly found in fall and winter (Bittner 2001) when predation is strongly reduced, whereas in fishless ponds and lakes, prevalence peaks in summer (Brambilla 1983; Vidtmann 1993; Stirnadel 1994). Duffy et al. (2005) linked the seasonal occurrence of *Spirobacillus* epidemics in several North American lakes to a drop in predation rate by bluegill sunfish.

Second, some diseases make their hosts more conspicuous through a reduction in transparency, thus increasing the likelihood of predation by visually hunting predators (Lee 1994; Yan and Larsson 1988) (P.T.J. Johnson, personal communication). Similarly, increased susceptibility to predation was reported for hosts carrying large loads of epibionts (Willey et al. 1990; Allen et al. 1993; Chiavelli et al. 1993; Threlkeld et al. 1993). Consistent with this, Willey and Threlkeld (1993) reported a reduction in the prevalence of clearly visible epibionts after stocking with fish. A prediction of this hypothesis is that parasites found in lakes with visually hunting fish should not make their hosts too visible or, if so, only in the terminal phase of infection. Consistent with this, the main parasites of *D. galeata* and *D. hyalina* in Lake Constance are hardly visible with the naked eye (Bittner et al. 1998, 2002; Bittner 2001). However, this hypothesis needs further careful examination. A twist to this hypothesis is that in turbid waters with low visibility, infected hosts may not have a reduced life expectancy relative to uninfected hosts (Decaestecker et al. 2005).

Third, fish predators are typically more common in larger ponds and lakes. A number of factors that go hand-in-hand with the size of lakes may limit the spread of parasites. Summer temperatures in larger water bodies may not rise as high as in smaller lakes in the same region, thus influencing parasite development or shortening the season during which parasites can occur (Ebert 1995). Furthermore, the sediment of larger, and in particular deeper, lakes may be a sink for parasite transmission stages. Parasite spores are known to rest in sediment, where they can be picked up by *Daphnia* (Ebert 1995; Decaestecker et al. 2002). In deep lakes, *Daphnia* are less likely to come in contact with lake sediment, thus reducing transmission rates. A prediction of this hypothesis is that parasites that rely exclusively on transmission from dead hosts are less likely to be found in deep lakes (see Chapter 8, Epidemiology, section on Transmission), as for example *P. ramosa*. In deep lakes, transmission from living hosts (e.g., gut parasites) may be much more important for the persistence of parasites.

Fourth, because *Daphnia* populations in lakes may not reach the density levels of pond populations, parasite transmission may be reduced. Two factors may account for this situation: a) lakes are often less nutrient rich (eutrophic) than ponds, so that lower rates of primary production may limit the maximum density of zooplankton populations; and b) predation by planktivorous fish may influence *Daphnia* density, and thus parasite transmission, negatively.

Thus, increased parasite mortality in *Daphnia* populations with fish predators and unfavorable conditions for parasite transmission in larger water bodies may act together to limit the spread of parasites in these *Daphnia* populations. One should keep in mind, however, that *Daphnia* communities in fishless ponds and those in lakes with fish are usually made up of different species. For example, whereas *D. magna* and *D. pulex* are more common in fishless water bodies, *D. galeata*, *D. hyalina*, and *D. cucullata* are typically lake-dwelling species. Therefore, the question of whether *Daphnia* populations in fishless water bodies have more parasites requires further critical scrutiny.

4.4 Conclusions and Open Questions

This survey of field studies clearly shows that parasites are abundant in natural *Daphnia* populations. It also shows that even under natural conditions, the harmful effect of parasites is usually clearly visible. Because field studies cannot address a number of factors, however, I will give, in the following chapter, an overview of experimental approaches that might tackle some of these remaining issues. For me, the key questions emerging from the survey of field studies are:

1. Which factors determine parasite richness in natural *Daphnia* populations? Why are parasites rare in some populations but very abundant in others? Are there fewer parasites in lakes with planktivorous fish?

2. It was often described that parasite prevalence increases in early summer and declines late in summer or fall. What determines the rise and decline of prevalence in these populations?

3. What role does *Daphnia* density play in parasite dynamics?

Chapter 5

The Effects of *Daphnia* Parasites on Host Fitness

Parasites use their hosts to foster their own needs, thus interfering with the hosts' survival and reproduction needs and creating a conflict of interest. In this chapter, I describe what is known about the damage that parasites inflict on *Daphnia*. It has been shown that many parasite infections reduce host fecundity and survival. Parasites may also influence other host fitness components, such as predator escape, body size, and sex allocation. Some parasites show specialized modes of action, such as castration or the induction of enhanced body growth. The degree to which parasites damage their hosts varies greatly among parasite and host species, parasite and host genotypes, and also depends on the interaction between the two. Environmental factors, such as temperature and feeding conditions, also play a role in the expression of disease symptoms.

5.1 Introduction

Part of the standard definition of parasitism is that parasites harm their hosts. As mentioned above, a number of field studies have shown that parasitized females often have reduced fecundity as compared with healthy (i.e., not parasitized) females. However, field data for some parasites have not revealed significant effects. Large environmental noise in the data and rather small parasite effects may render tests insignificant. Furthermore, if the host population is already in poor health (low food levels may also reduce the female's ability to carry eggs) or, alternatively, in very good health, the effect of the parasite may not easily be visible. Thus, it is not surprising that the apparent effect of parasites on host fitness varies if the same analysis is repeated in time or space (Yan and Larsson 1988; Bengtsson and Ebert 1998). Laboratory studies can reveal effects much more easily.

Because field studies usually cannot exclude the possibility that parasites infect hosts already weakened by other factors, such as poor nutrition, injuries, and inbreeding, their results must be considered with caution. Because laboratory experiments have demonstrated the clear fecundity costs of parasitism (see below), these confounding factors are unlikely to explain the bulk of the data. However, we need to be cautious when comparing field data across time, space, or species, because they are unlikely to reveal good quantitative data on parasite virulence.

The first attempts to demonstrate the effects of parasites under laboratory conditions used material from natural populations that had been brought to the laboratory for further observation (Green 1974; Brambilla 1983). Although these studies were able to observe differences between infected and uninfected females, they were not able to exclude various confounding factors. The in-

fected and the (apparently) uninfected females may have differed in life history traits (e.g., age or size) or may have already been in different conditions when they became infected. By the time infected animals were collected, the ages of their infections were also different. Although I do not believe that these confounding factors are highly critical when demonstrating some negative effect of parasites on host fecundity, they certainly interfere with testing the effects of the parasites on survival (see Chapter 3). Furthermore, with field-caught animals, one cannot quantitatively determine the strength of the effects. Thus, such experiments are not suitable for comparing the effects of parasites across space, time, or species.

A number of studies have attempted to test and quantify the effect of parasitism using proper experimental procedures with random allocation of females to different treatment groups and controlled infections. To my knowledge, every experiment of this sort revealed some negative effect of the parasite on their *Daphnia* hosts. Unfortunately, not all *Daphnia* parasites can be easily used for experimentation.

5.2 Effects on Host Fecundity and Survival

The two fitness components that are typically considered with regard to parasitism are host fecundity and survival. For both variables, drastic effects have been observed, and the degree of harm done to the host varies greatly. The costs of parasitism differ not only across parasite species but also among isolates of the same parasite and across environmental conditions (Ebert 1994b; Ebert 1998a, 2000a; Bittner et al. 2002). Figure 5.1 shows to what degree parasites differ in the damage they inflict on their host. Currently, the most harmful parasite tested is the White Fat Cell Disease, a bacterial infection in *D. magna* that severely reduces both host fecundity and survival (Ebert et al. 2000a). On the other end of the spectrum are the microsporidian gut parasites, such as *Glugoides intestinalis* and *Ordospora intestinalis*. These common parasites reduce host fitness by only 15% to 20%.

Across the entire range of observed effects, most tested parasites reduced both host fecundity and survival to a similar degree. Thus, parasites that drastically reduce life span also considerably reduce fecundity (fecundity of the living host relative to uninfected hosts of the same age), whereas parasites benign in their effect on survival were also benign in their effect on fecundity. In a first approximation, the reduction of both fecundity and survival may be seen as a general sign of host morbidity. In contrast to this pattern, *Pasteuria ramosa* shows a different course of infection. This bacterium first castrates its host (around 10 days after infection) but then allows it to live for many more days (over 40 days after infection). It has been speculated that this specific pathology is adaptive for *P. ramosa* (Ebert et al. 2004). Castrating the host allows *Pasteuria* to monopolize resources that the host would otherwise invest into reproduction. Early castration results in more parasite transmission stages.

5.2.1 Environmental Effects

Although the harm caused by parasites may depend on the environmental conditions, few studies have tested for environmental effects. Thus, no clear generalizations have emerged thus far. However, environment-dependent or condition-dependent virulence is certainly rather the rule than the exception. Survival and fecundity of *Daphnia* depend strongly on the abiotic and biotic environment (e.g., food quality and quantity, temperature, host density, presence and density of competitors, kairomones, and toxins), and some of these factors also influence the parasites. Thus, it is likely that these factors also influence the interactions between host and parasite.

Food Effects

The dependence of host fitness on the feeding conditions has been well documented for various *Daphnia* species. Lower food quantity or quality generally reduces fecundity but expands life span. The interaction between parasitic infections and the feeding conditions for the host has not yet been generally determined. Bittner et al. (2002) tested fecundity and survival of *Caullerya mesnili*-infected *D. galeata* in low and high food conditions. Although there was no significant difference in the survival of infected hosts, there was a strong effect on fecundity such that *C. mesnili* harms well-fed *D. galeata* more than poorly fed *D. galeata*. Infected *D.*

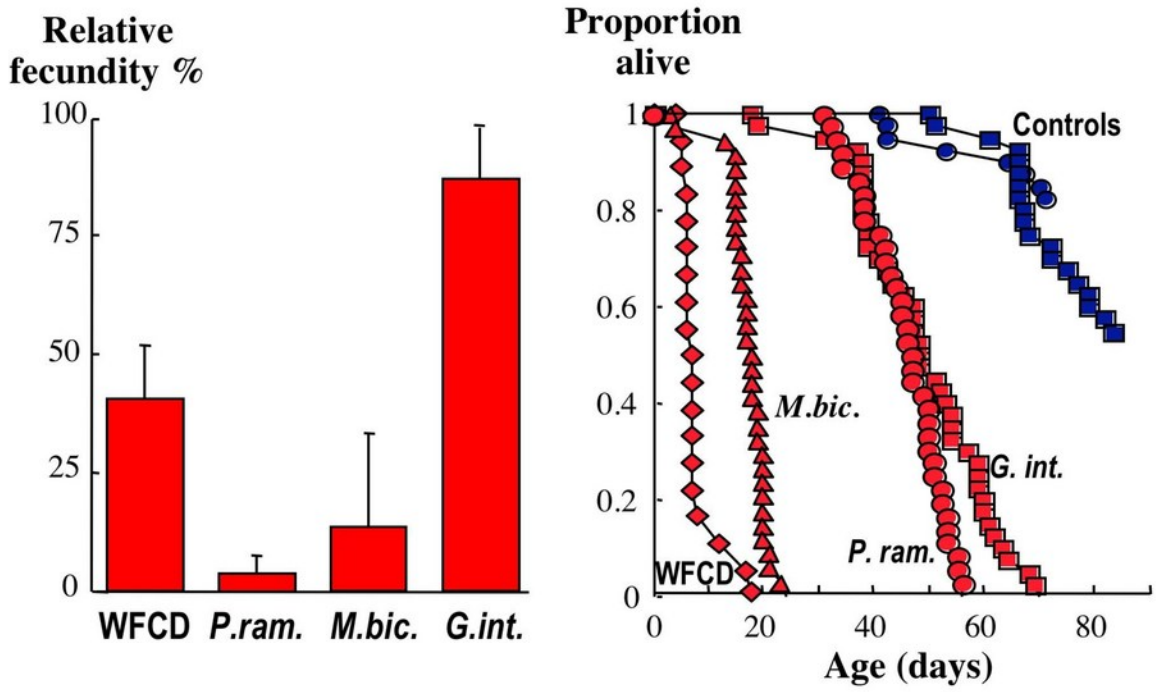

Figure 5.1 The effect of four parasite species on relative fecundity and survival of *Daphnia magna*. Relative fecundity is calculated as the total number of offspring of an infected female (until her death) relative to the total number of offspring of an uninfected female, assuming that the healthy female would have died the same day as the infected female. Thus, these relative fecundity measures are not confounded with different life expectancies of infected and uninfected females. *WFCD*, White Fat Cell Disease; *P. ram.*, *P. ramosa*; *M.bic.*, *Metschnikowia bicuspidata*; *G. int.*, *Glugoides intestinalis*. Redrawn and adapted from Ebert et al. (2000a).

galeata produced more eggs under low food conditions than under high food conditions. In contrast to the food study in *D. galeata*, a study on *D. magna* infected with *P. ramosa* found that well-fed infected hosts produced more eggs than poorly fed infected hosts (Ebert et al. 2004). Interestingly, the well-fed infected hosts also produced more *P. ramosa* transmission stages, indicating that good feeding conditions benefit both the host and the parasite. Both antagonists are possibly resource limited.

Temperature Effects

Healthy *Daphnia* mature earlier and at a smaller size and have a shorter life span when growing under conditions of higher temperature. Surprisingly little is known about the influence of temperature for the expression of disease in *Daphnia*. Duffy et al. (2005) reported anecdotally that *D. dentifera* infected with *Spirobacillus cienkowskii* survive longer at lower temperatures. Because usually everything with invertebrates takes longer at lower temperature, this observation may simply be the result of the hosts' and parasites' lower metabolic rates. A more complex relationship between temperature and disease expression was reported by Mitchell et al. (2005). They found that the negative effect of *P. ramosa* on *D. magna* fecundity was more benign when the temperature was lower. At a lower temperature, the parasite gained later control over host fecundity. The authors emphasize that this effect weakens parasite-mediated selection during part of the season. Furthermore, this parasite effect interacted both with host genotype and temperature such that clonal ranks in host fitness differed under different temperature conditions. This effect cannot be explained by the temperature dependence of metabolic rates. Altered rank orders of

host genotypes may have profound consequences for the evolution of host resistance. However, it is necessary to see these interactions in relation to the main effects and the seasonal dynamics of the disease to judge how evolution will be influenced.

Chemical Cues from Predators

Daphnia have been a workhorse for the study of phenotypic plasticity. In particular, their reaction to chemical cues released by predators (i.e., kairomones) has received a lot of attention. Lass and Bittner (2002) tested for interactions between the effects of two antagonists on *D. galeata*, the protozoan gut parasite*C. mesnili* and kairomones from planktivorous fish. They found no evidence for interactions between fish and parasite with regard to host fecundity and survival.

Dose Effects

Another environmental effect that influences the harm caused by parasites is the dose of transmission stages to which a host is exposed. Typically, higher doses go hand-in-hand with a higher likelihood of infection and with more severe damage to the host (Ebert 1995; Ebert et al. 2000b; Regoes et al. 2003; Ebert et al. 2004). Very high doses may even harm the host so much that the parasite is not able to complete its development before the host dies (Ebert et al. 2000b).

5.2.2 Genetic Effects

Genetic Variation among Hosts and Parasites

Parasite virulence varies across parasite isolates (strains, genotypes) and host clones. To my knowledge, every attempt to test for genetic variation within parasite-induced host damage in the *Daphnia* system has shown significant effects. Host clones originating from within or between populations differ in the degree with which they express disease symptoms, and parasite isolates vary greatly in the extent to which they cause damage to the same host clones (Ebert 1994a; Ebert 1998a; Little and Ebert 2000; Bittner 2001; Decaestecker et al. 2003). Furthermore, there are strong host clone x parasite isolate interactions: Within populations, the infectivity of *P. ramosa* depends strongly on the interaction between the *Pasteuria* and the *D. magna* genotypes (Carius et al. 2001) (Figure 5.2). The same is true if fecundity reduction is considered among infected females only (Carius et al. 2001). What maintains these high rates of within-population variation is not fully understood, but it has been suggested that antagonistic arms races play a key role in maintaining genetic variation for virulence and resistance (Hamilton 1980; Ebert and Hamilton 1996; Carius et al. 2001).

Genetic Variation across Populations and Local Adaptation

Genetic variation for parasite virulence is most pronounced across populations. This variation often follows a certain pattern, which is frequently discussed in the context of local adaptation (Kawecki and Ebert 2004). For four *D. magna* parasites, it has been shown that local parasite isolates cause more harm to their hosts than parasite isolates from other populations (Ebert 1994b; Ebert 1998a) (D. Refardt and D. Ebert, manuscript in preparation). These findings are consistent with the idea that parasites evolve local adaptation to the hosts they have encountered recently (Figures 5.3 and 5.4). Often (but not always) parasites that perform better in their local host than other foreign (or novel) parasites also perform better in their local hosts than in other hosts (Figures 5.3 and 5.5). Locally adapted parasites show not only higher levels of damage to their local hosts but also have higher levels of transmission-stage production (Ebert 1994b).

The finding of parasite local adaptation seems rather general in *Daphnia* systems but is not always found in other host–parasite systems. Some authors reported that hosts, rather than parasites, can be locally adapted (Morand et al. 1996; Kaltz and Shykoff 1998; Kaltz et al. 1999). It has been suggested that the key variable for the evolution of host or parasite local adaptation is the relative speed of evolution of the two antagonists (Gandon et al. 1996, 1997; Gandon 2002). Higher rates of mutation, recombination, and dispersal may facilitate local adaptation. Given these theoretical considerations and the finding that *Daphnia* parasites seem to be locally adapted, one may speculate that parasites of *Daphnia* usually have a higher evolutionary potential than their hosts.

A different approach to host–parasite interactions across populations is the question of how

Percentage infected

Pasteuria ramosa isolate \ *Daphnia magna* clone	A	B	C	D	E	F	G	H	I
A	78	89	94	11	0	11	17	0	83
B	83	89	89	61	11	56	50	16	89
C	0	78	61	94	55	0	0	50	33
D	0	89	67	94	50	0	0	61	28
E	0	78	55	83	39	0	0	39	28
F	0	78	0	0	0	56	33	0	0
G	33	78	44	11	0	67	44	0	44
H	0	67	39	83	22	0	0	16	22
I	89	89	100	0	0	0	0	0	100

Figure 5.2 Interactions between nine *D. magna* clones and nine *P. ramosa* isolates. Each cell gives the percentage of infected hosts for a given combination of host clone and parasite isolate. The table gives the average across two dose levels. All host and parasite genotypes were collected on the same day from the same pond. Modified after Carius et al. (2001) and Schmid-Hempel and Ebert (2003).

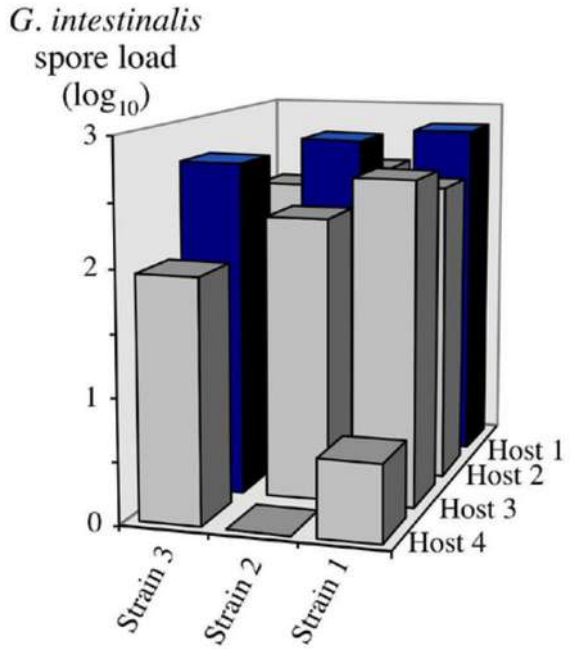

Figure 5.3 Local adaptation of *G. intestinalis* in *D. magna*. Strains of the microsporidium *G. intestinalis* from three different *D. magna* populations show the highest rates of spore production when infecting hosts from the their own native population (*blue columns*). The same strains in combination with hosts from four other populations (*gray columns*) produce much fewer transmission stages. Note the log10 scale for spore counts. Populations *Host 1*, *Host 2*, and *Host 3* are from southern England, population *Host 4* from southern Germany. For more information, see Ebert (1994b).

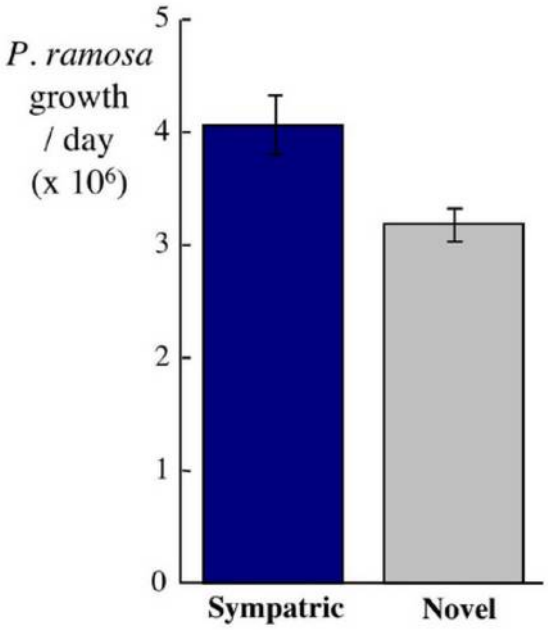

Figure 5.4 Local adaptation of *P. ramosa* in *D. magna*. Three different strains of the bacterium show the highest within-host growth rates when infecting hosts from their own native population (*blue column*) (mean and standard error). Other *Pasteuria* isolates (*Novel*) tested in the same host clones (*gray column*) have lower growth rates. For more information, see Ebert (1998a).

much a dispersing host suffers when it encounters a locally adapted parasite in a novel population. Note that this question is different from the question about parasite local adaptation. Kawecki and Ebert (2004) explain these differences in full detail. If parasites are locally adapted and thus cause more harm to their local hosts, a host that migrates into such a population should, one expects, suffer less on average from the local parasites than the local hosts. This observation has been reported in several experiments (Ebert 1994b; Ebert et al. 1998; Altermatt 2004). It is important to note that although this pattern is found when averaging across several host–parasite combinations, occasionally a host in a novel combination is much more affected by the new parasites than expected (Ebert 1994b). These instances are likely to be exceptions, but they may have profound consequences, because they may be the beginning of a devastating epidemic. Further information about the evolution of virulence can be found in a number of reviews (Bull 1994; Ebert 1998a, 1999; Ebert and Bull 2003).

5.3 Parasite Effects on Other Host Traits

Besides fecundity and survival, parasites may influence other aspects of host fitness, few of which have been studied. *G. intestinalis* (formerly *Pleistophora intestinalis*) reduces adult growth in its host *D. magna* (Ebert 1994b). The strength of this effect was shown to depend both on host clone and parasite isolate, with local parasite isolates having

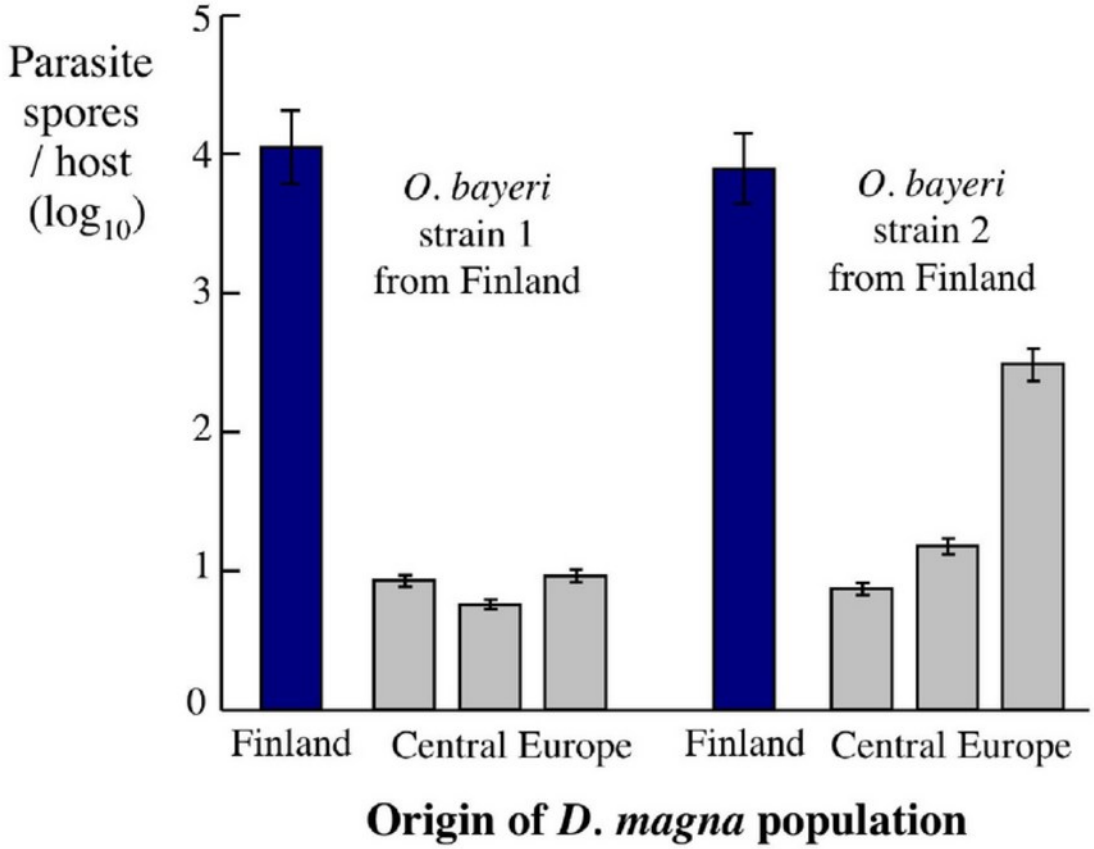

Figure 5.5 ***O. bayeri*** **spore production in clones of its native** ***D. magna*** **population and in clones from three other** ***D. magna*** **populations**. Two strains of *O. bayeri* originating from two islands of a rock pool metapopulation of *D. magna* in southern Finland were tested in combination with their own and three central European populations of *D. magna*. Means (across clones) and standard errors are given (between 4 and 11 clones were used per host population). For more information on "Material and Methods", see Mucklow et al. (2004).

the strongest effect. Lass and Bittner (2002) showed that *C. mesnili* reduced the adult growth of its host *D. galeata*. In contrast, *P. ramosa* causes its host *D. magna* to grow to an unusually large size (Ebert et al. 1996, 2004). This form of parasite-induced host gigantism may be adaptive for the parasite, as larger hosts result in more parasite spores being produced (Ebert et al. 2004).

Parasites may also influence aspects of their hosts' sexual life cycle. For example, they may reduce the hosts' likelihood of finding mates or may increase or decrease the frequency with which a female produces ephippia and male offspring. Furthermore, vertically transmitted parasites may influence the survival of their host during resting (Lass and Ebert 2005).

5.4 Parasites May Influence Predation on Their Hosts

The potential effect that parasites have on host–predator interactions is also important. Parasites may lower the ability of their hosts to escape predators; infected hosts may swim and react more slowly than healthy hosts, for example. The sometimes dramatic visual effect that parasites have on *Daphnia* may even directly increase the hosts' attractiveness to visually hunting predators (Yan and Larsson 1988; Lee 1994; Duffy et al. 2005).

Lass and Bittner (2002) tested for more indirect effects of parasites on host–predator interactions. They tested whether hosts are less able to show adaptive phenotypic changes against predators when exposed to *C. mesnili*. Their experiments revealed no significant interactions between parasite and kairomon-induced life history changes. They concluded that this is because the host's adaptive response against fish predators changes life history traits expressed early during the host's life, whereas the parasite affects its host during later stages.

On the other hand, one can imagine that parasites alter their host's behavior so that hosts more effectively protect themselves from predators, e.g., by altering vertical migration. This may still be disadvantageous for the host because the parasite's interest is in host survival, while the host has to trade-off protection from predators against other fitness components, such as reproduction. Lee (1994) and Fels et al. (2004) showed that various parasite species influence the depth selection behavior of *D. magna*. Infected hosts stay deeper in the water than uninfected controls. It is not clear, however, whether this is adaptive for the host, the parasite, both, or none.

An extreme example of altered predator exposure would be a case in which the parasite manipulates its host's behavior to facilitate it own transmission to the next host. To my knowledge, none of the described unicellular parasites of *Daphnia* has a known second host, although this option has been speculated (Mangin et al. 1995). However, the macroparasites (helminth) parasites of *Daphnia*, which have not yet been extensively studied, have second hosts and may well manipulate their hosts to their own advantage (Stammer 1934; Green 1974; Schwartz and Cameron 1993).

5.5 Conclusions and Open Questions

There is little doubt that parasites of *Daphnia* and other Cladocerans are generally harmful. Occasional reports of "nonsignificant" effects of parasites have to be considered in the light of low statistical power or large environmental noise. Thus far, every species tested under controlled conditions proved harmful. What I find more interesting than the fact that the parasite harms its host are questions regarding the covariables of the degree of harm. There are a number of interesting questions about this:

1. Why are some parasites more harmful than others? What role does the parasite's taxonomic position play for its virulence? What role does the mode of transmission play? What role does the specific tissue infected play?

2. Are there further hidden costs of parasitism in *Daphnia*? For example, do parasites influence mate choice during sexual reproduction? Do parasites influence the survival of resting eggs?

3. Does inter- and intra-specific competition of parasites influence virulence?

Chapter 6

Host Adaptations against the Costs of Parasitism

As parasites harm their hosts, the host may counteradapt, reducing the fitness costs of parasitism. Here I summarize the little we know about the ways *Daphnia* adapts to lower the costs of parasitism. One known example is that *D. magna* matures earlier in the presence of infections. I further discuss what is known about induced defense and the evolution of resistance in *Daphnia*. The chapter closes with a discussion of the limits of host resistance. Thus far, no evidence for a cost of defense has been found in *Daphnia*.

6.1 Introduction

Parasites harm their hosts to foster their own needs. As studies thus far have shown, this damage varies across host clones, suggesting the presence of genetic variation among hosts for resistance or the expression of disease. This genetic variation for fitness-related traits may bring about different reproduction and survival rates among host genotypes, so that host clones that suffer less from parasitism increase their numerical representation in the host population. If at least part of the genetic variance for fitness is based on additive genetic variance, the host population may adapt to counteract parasites even across the sexual life cycle, i.e., even after the gene combinations in the clones are recombined into new genotypes.

Thus far, we have only a few clear examples of *Daphnia* hosts adapting to parasitism. There are two main problems with detecting host adaptations. First, if host adaptations lower parasite fitness (which is often but not necessarily always the case), parasites may rapidly evolve counteradaptations that reduce the effectiveness of the host adaptations and may make them invisible. A prediction of this theory is that host adaptations are more likely to be found in the presence of coevolving parasites if the adaptation benefits the host greatly but poses little or no disadvantage to the parasite. For example, the reduction of "unnecessary virulence", i.e., parasite-induced damage to the host that has no benefit for the parasite, could be an easily detected host adaptation (in novel, not yet coevolved, host–parasite associations, such unnecessary virulence is sometimes observed). Second, the adaptive value of host traits expressed in the presence of parasites may be difficult to judge because they stem from the interaction between two organisms and may or may not be beneficial to both (Moore 2002). For example, is the *Daphnia's* parasite-induced change in diel vertical migration (Fels et al. 2004) beneficial for the host, the parasite, both, or none?

Host adaptations to parasites may be observed at several levels. The most impressive examples are those where a trait is expressed only in exposed or infected individuals and confers a benefit compared with individuals that do not express

this trait. Examples of such phenotypic plasticity are early maturity and reproduction in exposed or infected females (Minchella and Loverde 1981). The same adaptation may, however, be constantly expressed within a host population (Jokela and Lively 1995). This may be beneficial if the host population suffers high rates of infection or if the constant expression of the adaptation has no associated costs in the absence of the parasite. Investigating constantly expressed host adaptations requires a comparison across host demes (populations) with variable degrees of parasitism and may require correction for common ancestry (Felsenstein 1985). If adaptations are thought to be host species or taxon specific, a comparative approach to the species or even to a higher taxonomic level may be required.

To verify that a host trait originates from host adaptation, one must carefully analyze the costs and benefits of this trait for both the host and the parasite. In some cases, this is rather straightforward, e.g., encapsulating and killing the parasite is obviously a host adaptation. It is, however, less simple in other cases, such as the enhanced growth of infected hosts, which some have suggested is adaptive for parasites (Baudoin 1975; Sousa 1983; Ebert et al. 2004), whereas others have argued that it is adaptive for the host (Minchella 1985; Ballabeni 1995). Below I discuss a few examples where there is good evidence that the traits observed are adaptive for the host.

6.2 Changes in Life History Traits

Although parasite-induced changes in host life history traits are frequently observed, most of them stem from the negative consequence of parasite exploitation (e.g., reduced fecundity and survival) and are not a host adaptation. The life history change that has received the most attention in various systems is the early reproduction of hosts that are exposed to or infected with parasites (Minchella and Loverde 1981; Jokela and Lively 1995). Early maturation has also been found in connection with two *Daphnia* parasites. In most *D. magna* clones, early maturation occurs when the host is infected early in life with the castrating bacterium *Pasteuria ramosa* (Figure 6.1) (Ebert et al. 2004). This change in life history has been shown to benefit the host by increasing its lifetime reproductive success relative to infected hosts that do not show this response. Furthermore, early host maturation and reproduction harm the parasites by lowering the hosts' transmission stage production because resources invested into host reproduction are not available for the parasite (Ebert et al. 2004). Likewise, Chadwick and Little (2005) observed that *D. magna* shift their life-history strategy toward early reproduction when infected with the microsporidium *Glugoides intestinalis*.

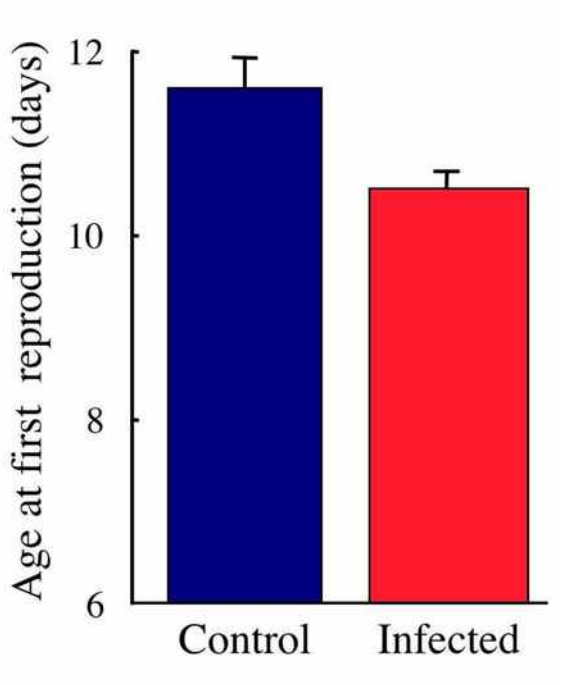

Figure 6.1 ***D. magna*** **matures earlier when infected early in life with** ***P. ramosa***. Means across two food levels are shown. Modified from Ebert et al. (2004).

6.3 The Evolution of Host Resistance

Every *Daphnia* population tested for genetic variation in resistance has revealed high levels of clonal variation. Thus, *Daphnia* populations are probably under permanent selection for resistance. That they do not evolve efficient resistance suggests that the parasites have a high potential for evolv-

ing counter-resistance. However, clonal variation for resistance itself does not prove adaptive evolution. Experimental evolution has demonstrated that hosts do not evolve only in the presence of parasites but also that evolution proceeds very quickly.

Capaul and Ebert (2003) tested the extent to which parasite-mediated selection by different parasite species influenced competition among clones of the cyclic parthenogen *D. magna*. We monitored clone frequency changes in laboratory microcosm populations consisting of 21 *D. magna* clones. Parasite treatments (two microsporidians, *G. intestinalis* and *Ordospora colligata*) and a parasite-free control treatment were followed over a 9-month period. Significant differences in clonal success were found among the treatments as early as one month (about two to three *Daphnia* generations) after the start of the experiment (Figure 6.2). The two parasite treatments differed not only from the control treatment but also from each other. The consistency of clone frequency changes across the replicates within treatments indicated adaptive evolution specific to the parasites used. The results suggest that parasites may influence microevolution in *Daphnia* populations even during short periods of asexual reproduction. A similar design was used by Haag and Ebert (2004), although in this study *D. magna* clones competed in mesocosms under outdoor conditions for one summer season. We also found rapid and significant changes in clonal composition across treatments.

These studies clearly demonstrate that microevolutionary change in *Daphnia* populations can be observed within short periods of time and that they are specific to the parasite treatment used. They did not, however, allow us to identify which traits were selected for, although it is reasonable that resistance to parasites played a role. In a follow-up experiment, we tested whether, under natural conditions, *D. magna* host populations showed higher levels of resistance after 2 years of evolution, including sexual recombination and diapause. The results showed that the hosts that evolved in the presence of the microsporidium *Octosporea bayeri* had a higher fitness than the controls in the presence of the parasites (M. Zbinden et al., manuscript in preparation). Fitness in this experiment was measured in a competition experiment that mimics the conditions under which the *Daphnia* evolved.

6.4 Induced Defense

A cost-effective way of protecting against invaders is to launch a defense mechanism only when challenged by a parasite or only under conditions where there is an increased likelihood of contracting disease. Little is known about the immune response of lower crustaceans, and because of their small size, it is difficult to study the physiology of the immune system. This will change when more genetic data become available (see, for example, Little et al. 2004).

A relatively easy way to investigate part of the immune system is through the prophenoloxidase (PO) system, which has received a lot of attention among ecologists interested in immunology, although it is not clear whether this system is more important than other aspects of the invertebrate immune system. The PO system has been used for testing hypotheses about induced defense; however, because it is believed to play a role in protecting invertebrate hosts from infections (Söderhall 1999). Mucklow and Ebert (2003) studied the system for *Daphnia* and showed that wounded *D. magna*, which presumably have a higher likelihood of contracting infections, have an up regulated PO activity. PO activity was also higher in well-fed animals than in poorly fed animals, suggesting that the expression of a high level of PO activity is costly. However, in a follow-up experiment, Mucklow et al. (2004) did not find that wounded *D. magna*, which presumably up regulated their PO activity, showed increased levels of resistance against the bacterium *P. ramosa*. Thus, a generalized induction of the PO system does not seem to reduce the risk of contracting disease.

Little et al. (2003) showed that induced defense may be highly specific. The hallmark of the vertebrate immune system is an acquired response against specific antigens. Memory cells resulting from a primary infection enhance the proliferation of antibodies during secondary infection. For invertebrates, an adaptive immune system with an immune memory has not yet been observed. Thus, invertebrates were believed to be naive at each new encounter with parasites. Little et al. (2003) found evidence for acquired immunity in *D. magna* infected with *P. ramosa*. Immunity was shown to be parasite strain specific to some degree. Host fitness was enhanced when the host was challenged by a *P.*

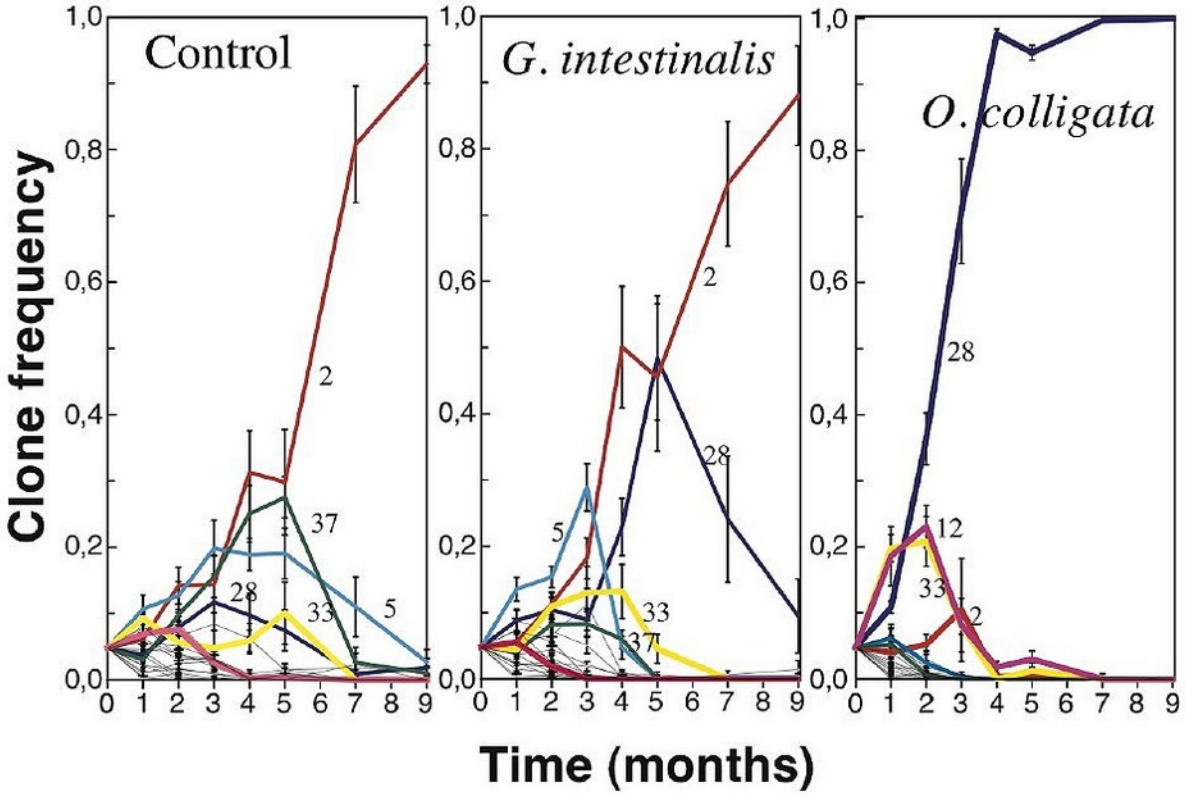

Figure 6.2 Clonal competition among 21 clones of *D. magna* in the absence (*Control*) or presence of two microsporidian parasites (*G. intestinalis* and *O. colligata*). Only 6 of the 21 clones are shown in color. Clones that did not contribute significantly to the overall dynamics are shown in *grey*. The small numbers are clone identifiers. Redrawn from Capaul and Ebert (2003).

ramosa strain that its mother had experienced relative to cases when mother and offspring were challenged with different strains. If this finding holds in general for *Daphnia* and other invertebrates, it would open a huge field of research for both the molecular mechanisms of acquired resistance and its evolutionary and ecological consequences.

6.5 Limits to the Evolution of Host Counter Adaptations

The evolution of defense against natural enemies may not come for free, i.e., there may be a trade-off between resistance (and/or tolerance) to parasites and other fitness components (Kraaijeveld and Godfray 1997). Such trade-offs may prevent the fixation of resistant genotypes and therefore could slow down or even prevent the evolution of resistance. This may explain why genetic polymorphism is maintained for resistance in the wild. Obviously, if the defense is more costly than the damage caused by the antagonists, it will probably not evolve. Even small costs of defense may slow down or hinder the evolution of defense because the costs may be paid permanently, whereas the enemies are encountered with only an uncertain likelihood. It may never pay off to invest in resistance against a rare parasite.

6.5.1 Costs of Resistance

Little et al. (2002) tested for the costs of resistance in a number of experiments with *D. magna* and *P. ramosa* but failed to detect any evidence for these

costs. They tested whether resistant host clones have a reduced competitive ability or pay costs in the form of altered life history characteristics (e.g., delayed maturation, lower fecundity) in the absence of the parasites. They concluded that a cost of resistance is unlikely to explain the maintenance of genetic variation in the *D. magna* –*P. ramosa* system.

6.5.2 Trade-offs between Defense Options

Decaestecker et al. (2002) looked for a different form of cost of defense by studying habitat selection behavior, which is an important component of the *Daphnia's* predator-avoidance strategy. The evolution of this behavior is often explained as a trade-off between avoiding antagonists and acquiring resources. Negatively phototactic clones suffer less from visually hunting predators because they reside deeper in the water column during the daytime. However, this behavior increases the risk of infections because they are exposed to pond sediments containing parasite transmission stages. Positively phototactic clones, which are at a higher risk of predation, are less exposed to parasite spores in the sediment and consequently suffer less from parasitic infection. The authors showed that the increased risk of infection also holds when the animals change their phototactic behavior upon exposure to chemical cues from fish. This study highlights a substantial cost of predator-induced changes in habitat selection behavior. Such trade-offs may explain genetic polymorphism for habitat selection behavior in natural *Daphnia* populations.

Speculating along the same lines, one may postulate that hosts have to trade off alleles for resistance against each other. If resistance requires certain alleles at a locus, the possession of one allele precludes the possession of another allele. Decaestecker et al. (2003) tested 19 *D. magna* clones for resistance against five parasite species to discover whether resistance against different species is traded off against each other. They were unable to find evidence for such trade-offs, although they found strong evidence for host–clone times parasite–species interactions. The same observation was reported by Carius et al. (2001) when they tested various combinations of *D. magna* clones with isolates of *P. ramosa*. Thus, the current evidence suggests that there is no trade-off for resistance against different isolates of parasite species.

6.6 Conclusions and Open Questions

The few examples given in this chapter show that *Daphnia* have evolved various ways of reducing the costs of parasitism. Some of these are likely to be phylogenetically old (evolution of immune response; PO system), whereas others seem to evolve very rapidly. The latter may play an important role in the host–parasite arms race (Ebert and Hamilton 1996; Ebert 1998a; Schmid-Hempel and Ebert 2003). Many fascinating questions about host adaptations remain unexplored, however:

1. What is the underlying genetic system for the interactions between hosts and parasites?
2. How many genes are involved in host resistance?
3. Are there costs for resistance? What do these costs look like?
4. Why is there no super-resistant host genotype?

Chapter 7

Host Range of *Daphnia* Parasites

In this chapter, I summarize what we know about parasite host ranges and host specificity. I outline the ecological, epidemiological, and taxonomic considerations relevant for assessing host ranges and discuss the problems with describing host ranges in field studies, where the investigation of host ranges is hampered by low statistical power, and laboratory studies, where the absence of evidence is not necessarily evidence for absence. I argue that *Daphnia* parasites are generally more specific than thought previously.

7.1 Introduction

Every parasite has a host, but no parasite can infect all potential hosts. Moreover, parasites are usually very limited in the number of host species they are able to infect. Thus, in describing a parasite's host range, one defines its niche. This description usually resembles a list of host species that a parasite is able to infect. The description of the host range usually does not distinguish the degree to which a parasite is able to infect a host and which hosts it prefers to infect. Therefore, the host range, presented as a list of potential host species, cannot tell us much about the evolution and ecology of a parasite, nor about its consequences for the host. Nevertheless, the host range can be, at least locally, a useful tool for identifying certain parasite species and can sometimes even help identify host species by the presence of their specialist parasites.

Host specificity describes the degree to which a parasite is a specialist. This term is often used together with host range, such that a wide host range indicates a low specificity. However, although host range is often described as a list of potential host species, specificity is often used to describe host–parasite associations from a more quantitative perspective, e.g., which hosts are preferred. Host specificity is very important for both ecological and evolutionary aspects of host–parasite interactions. Biologically speaking, any difference in the degree to which a parasite is associated with different host species indicates some degree of specificity. Thus, specificity may range from extreme forms, such as the ability of a parasite to infect only certain members of one host species, to slight differences in the degree to which the parasite infects or harms different host species. To gain a deeper understanding of a particular system, it is also helpful to take into account the consequences of specificity for the host and for the parasite.

For ecological and evolutionary questions, it is also important to consider from whose point of view one considers specificity. For a parasite, a host is suitable if the parasite is able to reproduce in and transmit from this host species. Hosts that do not allow for secondary infections are of little relevance for the parasite's host range, although the interaction may still be detrimental to the host. A host's perspective is different. A host is part of a host range if it can be infected by the parasite, even if the parasite does not do well in this host. Ecologically, this difference in perspective can be

important when considering the spread of parasites and the coexistence of host species.

7.2 Understanding Host Ranges of *Daphnia* Parasites

In his review of "Parasites and Epibionts of Cladocera", Green (1974) stated that, "It seems unlikely that many of the parasites and epibionts of Cladocera will prove to be highly specific in their host preference" (page 490). Although I tend to agree with this statement regarding epibionts (for example, Gilbert and Schröder 2003), I think that we lack the necessary data to conclude that parasites are usually unspecific. Some species (e.g., the microsporidium *Octosporea bayeri*) are known to be highly host species specific. It is clear that we need more studies to reach a general conclusion on this point.

We currently know little about the host ranges and host specificity of *Daphnia* parasites. When investigating potential hosts, one must consider a number of questions whose answers are not as clear-cut as the relative ease of studying host specificity in *Daphnia* might suggest. However, this complexity allows us to dig deeper into aspects of host range evolution, which is certainly a very fascinating topic in evolutionary parasitology. Before I discuss how to estimate host specificity, I will briefly outline some problems that are important from an evolutionary perspective.

Results from field and laboratory studies suggest that infections are often highly dependent on the host clones, on the population from which the hosts and parasites were collected, and on the ecological settings in which the data were gathered. Thus, statements about host specificity must take into account variation within and between species and even within populations. For example, *Pasteuria ramosa* shows very strong host clone–parasite isolate interaction. Within populations, different clones of *D. magna* vary widely in their susceptibility to different isolates of *P. ramosa* and vice versa (Carius et al. 2001) (Figure 5.2). Furthermore, *P. ramosa* can be locally adapted to its host population (Ebert et al. 1998) such that it grows best in hosts from the population from which it was isolated. On the other hand, *P. ramosa* is able to infect several *Daphnia* species and even other Cladocerans (Green 1974; Stirnadel and Ebert 1997). Thus, a conservative approach would classify *P. ramosa* as being rather unspecific with regard to the host species it is able to infect. *P. ramosa* is, however, highly specific in its interactions with particular host genotypes, in seeming contrast to its apparently wide host range. The reason for this discrepancy is currently not clear. One possibility is that the *P. ramosa* species is composed from many lines, each with a narrow host range, but all together having a very wide range. However, the alternative, that single *P. ramosa* genotypes are able to infect only certain host clones within a species as well as certain clones from other species (narrow within host species range but wide range across host species), cannot be excluded, although it seems to go against the intuition of many evolutionary parasitologists. These two hypotheses can be easily distinguished experimentally.

From an ecological perspective, host specificity may not only be defined by the ability of the parasite to infect a host but also by its effect on the host. For example, *Caullerya mesnili* is able to infect *D. galeata* and *D. hyalina*. However, in *D. haylina* it is rather benign, whereas it is highly virulent in *D. galeata* (Bittner 2001). Thus, virulence is specific to *D. galeata*.

Finally, it should be noted that literature reports of the same parasite species in different *Daphnia* species or the same parasite species in different localities are often not very trustworthy unless they are combined with detailed taxonomic and/or molecular investigations. Because parasites are usually not very rich in morphological characters, it is easy to pool different species into one taxon. It seems likely to me that many currently described parasite species will turn out to be a group of species.

7.3 How to Describe and Test Host Ranges

A practical way to judge a parasite's ability to infect different host species based on field data is to compare the prevalence of infections when both hosts are present in the same lake or pond. This method, however, is rather conservative. Bittner (2001) used it to assess the host specificity of seven parasite species, all of which appeared to be some-

what host specific. Using the appropriate statistical test, she found firm support for specificity for only one parasite species. Some of the other parasite species that had appeared to be host specific occurred only rarely or were found only when the other host species was absent, which made a proper comparison impossible. Stirnadel and Ebert (1997) used the same method to classify the specificity of parasites from three populations, each of which had *D. magna, D. pulex*, and *D. longispina* occurring in sympatry. *Gurleya vavrai* was the only parasite unambiguously classified as specific (infecting *D. pulex* and *D. longispina* but not *D. magna*), whereas *Metschnikowia bicuspidata, P. ramosa*, and an unknown fungal parasite were clearly able to infect all three *Daphnia* species. Some parasites appeared specific in one pond but unspecific in another pond. Whether these findings are explained by local genetic differences or misidentification of parasites (presence of cryptic species) is not clear. Again, low statistical power for the less common parasites prevented us from reaching firm conclusions for a number of parasite species.

A slightly different approach was used by Bengtsson and Ebert (1998) and by Ebert et al. (2001) in a *Daphnia* metapopulation context. In these studies, specificity was judged on replication across numerous rock pool populations. If two *Daphnia* species occur together in a number of rock pools and one of them is significantly more often infected by a certain parasite species, one may conclude that the parasite is specific to this host. Bengtsson and Ebert (1998) found that none of the parasite species they observed were specific to one of the two host species. However, the microsporidium *Larssonia* sp. showed consistently higher prevalence in *D. pulex* than in *D. longispina* whenever both host species were sympatric in a rock pool. Furthermore, *Larssonia* sp. seemed to have a stronger fitness-reducing effect on *D. pulex* than on *D. longispina*. Ebert et al. (2001) found that White Fat Cell Disease and two microsporidians, *Ordospora colligata* and *Octosporea bayeri,* were specific to *D. magna* (not infecting *D. longispina* and *D. pulex*). *Spirobacillus cienkowskii* and *Larssonia* sp. (possibly the same species as in Bengtsson and Ebert 1998) infected all three *Daphnia* species. The numerous epibiont species found in rock-pool *Daphnia* infected all available host species and were even seen to colonize hosts from other taxa, e.g., insect larvae. For the other parasites, no clear statement could be made.

7.4 Conclusions

The available evidence suggests that *Daphnia* parasites differ strongly in the degree to which they are associated with different host species or host clones. Host ranges indicate only the number of host species a certain parasite species is able to infect and can include anything from one to many host species, as well as hosts of different genera or families. Considering specificity from a quantitative perspective, which takes into account quantitative differences in the susceptibility of hosts, every parasite probably shows some degree of specificity. Because the current statistical methods are conservative and some parasites are rare, I believe that more detailed investigation will reveal more examples of specific parasites. Laboratory experiments can best elucidate the host range of parasites with well-defined transmission mechanisms. Parasites that do not transmit horizontally under controlled conditions must be studied by field observation.

Chapter 8

Epidemiology

Epidemiology of infectious diseases attempts to describe the patterns and processes by which diseases are distributed in the host population. Here I present what is known about the transmission of *Daphnia* parasites, about the factors that influence transmission, and how they work together in shaping parasite dynamics. I further discuss two general models of parasite epidemiology, one for *Daphnia* populations in fishless ponds, another for *Daphnia* populations in lakes with planktivorous fish.

8.1 Transmission

In a parasitological context, epidemiology is the study of infectious diseases and disease-causing agents at the population level. It seeks to characterize the patterns of distribution and prevalence of the disease and the factors responsible for these patterns. In a more applied context, it also strives to identify and test prevention and treatment measures. The key factor to understanding the epidemiology of diseases is to understand transmission, or the movement of parasites from one host to the next.

In the following, I focus on four aspects of transmission: the mode of transmission, the survival of transmission stages, the uptake of transmission stages from sediments, and the factors that may limit transmission in natural populations.

8.1.1 Modes of Transmission in *Daphnia*: Parasite Systems

An important component of epidemiology is the parasite's mode of transmission, or how it moves from one host to the next. Unfortunately, surprisingly few scientific reports include information on parasite transmission. To my knowledge, the first description of a plankton parasite life cycle that tested mode of transmission was the description by Chatton (1925) of the amoeba *Pansporella perplexa* in *Daphnia pulex*. This parasite is transmitted between hosts via waterborne infective stages, which are released from infected hosts and are ingested by the same or other host individuals during filter feeding.

The modes of transmission of *Daphnia* parasites can be grouped into four types; these do not, however, exclude each other, because some parasites can be transmitted by more than one method (Figure 8.1).

Horizontal Transmission from the Living Host

This form of transmission is the typical mode of transmission for many human and livestock infectious diseases. Infected hosts release infective particles, which then infect other hosts (Figure 8.1A). Influenza and measles are typical examples. This mode of transmission is frequently found among *Daphnia* parasites, particularly gut parasites, but also epibionts. *Daphnia* parasites that use this mode of transmission are the amoeba *Pansporella perplexa*, the microsporidia *Glugoides intestinalis* and

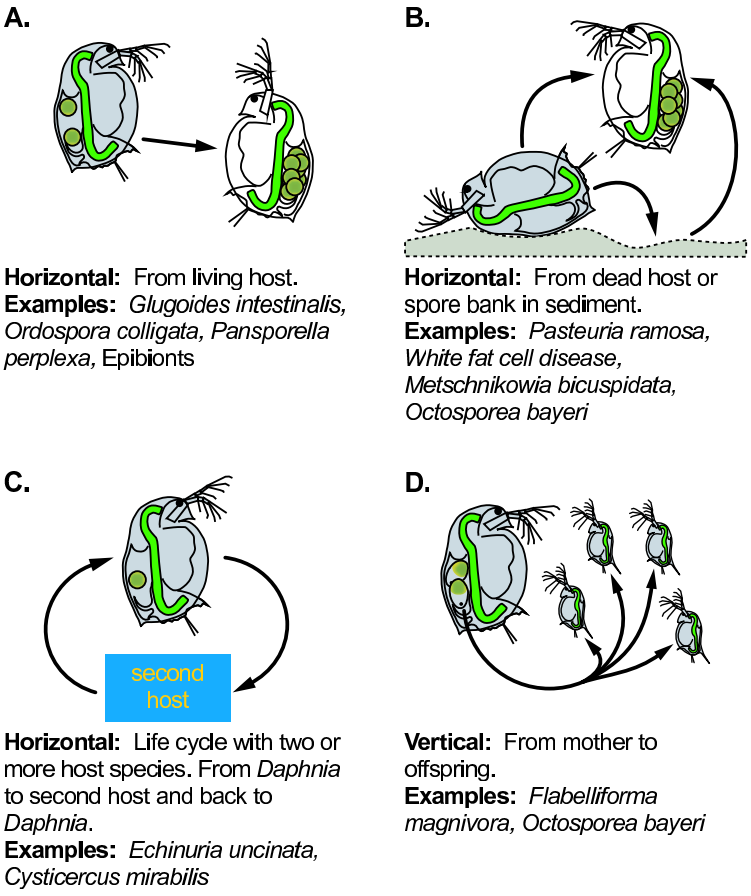

Figure 8.1 The four typical modes of transmission for *Daphnia* parasites. The *arrows* indicate the direction of parasite transmission. The *blue box* in C represents a second host, which is likely to be a duck for the two helminth parasites listed but may be an invertebrate for the other parasite species. Drawing by Dita B. Vizoso.

Ordospora colligata, and the protozoan *Caullerya mesnili*. With these gut parasites, infected hosts carry comparatively few transmission stages at any one time (compared with the parasites falling into the next group), although they may produce many transmission stages during the lifetime of an infection.

To the best of our knowledge, all of the parasites in this category enter their hosts with the food. Food uptake by *Daphnia* is through filter feeding, and the rate at which *Daphnia* filter their food therefore plays an important role in the spread of a disease (Fels 2005).

Brood parasites have been observed to occur in numerous *Daphnia* populations. These are typically transmitted from one living host to the next. The most devastating ones are certain fungi, which kill the entire brood while it is developing in the brood chamber. Brood parasitic copepods may also be listed here. In contrast to the other parasites in this group, they actively search for their host and enter the brood pouch from behind.

Horizontal Transmission from the Dead Hosts and Sediments

Parasites that infect tissues other than the host gut or body surface may have more problems leaving their hosts. These parasites often produce many transmission stages that are only set free after the host's parasite-induced death (Figure 8.1B). By the time of the host's death, these obligate killers (Ebert and Weisser 1997) may produce up to 100 million transmission stages, which are all released at once. Killing the host to achieve transmission is common among insect parasites (many viruses and bacteria) but seems uncommon among parasites of vertebrates (*Alien*, the deadly extraterrestrial from the movie with the same name, which killed the human crew of a spaceship, is the only exception known to me). Examples of *Daphnia* parasites with this mode of transmission include the blood parasitic bacteria *Pasteuria ramosa* and White Fat Cell bacterium, the yeast *Metschnikowia bicuspidata*, and the microsporidium *Octosporea bayeri*.

Once set free from the dead host, the spores of parasites that kill obligately must reach another host to achieve transmission. If the pile of transmission stages left by a decaying host is stirred up, spores may be suspended in the water and infect filter-feeding hosts. Some *Daphnia* species tend to browse over substrates and thus come into contact with very high local concentrations of transmission stages in the sediments, which may then be ingested.

Although we do not currently know how *P. ramosa* enters the host, the closely related parasite *P. penetrans* enters its nematode host through the cuticula (Preston et al. 2003), which may be the same route used by *P. ramosa*. In this case, it would be the only known parasite of Cladocera that does not enter the host with the food.

Despite the apparent advantage of killing the host early to achieve transmission, parasite virulence with transmission from dead hosts varies greatly and ranges from rapid killers (e.g., White Fat Cell Disease) to parasites that have only a modest impact on host survival (e.g., *P. ramosa, O. bayeri*). The reasons for this large variation may be found in the specific biology of the parasites (Ebert and Herre 1996; Ebert and Weisser 1997), but our knowledge about the evolution of virulence is still rather rudimentary.

Horizontal Transmission with a Two-Host Life Cycle

A number of parasites cycle through two or more host species to complete their life cycle (Figure 8.1C). Among the Cladocera, however, there are only a few known examples of parasites with multi-host life cycles. This is surprising, because life cycles with two hosts are well known among parasite systems where at least one host lives in freshwater, including a number of human parasites, such as the medina worm (*Dracunculus*) and *Schistosoma*. The only known examples of *Daphnia* parasites with a two (or more) host life cycles are the nematode *Echinuria uncinata*, the cestode *Cysticercus mirabilis* (Green 1974), and an undescribed trematode parasite of *D. obtusa* (Schwartz and Cameron 1993). It is possible, however, that some of the microsporidian parasites of *Daphnia* that appear untransmissable in the laboratory, such as *Flabelliforma magnivora*, have a second host (Mangin et al. 1995) (Mangin et al. called this species *Tuzetia* sp.).

Although the uptake of the parasites by the second host species is likely to happen via deliberate or accidental ingestion of infected *Daphnia*,

the uptake of the parasite by *Daphnia* is currently unknown for all helminth parasites. It is possible that *Daphnia* pick up, with their food, transmission stages that are released from the second host.

Vertical Transmission

Vertical transmission describes the movement of a parasite from the mother (seldom the father) to the offspring (Figure 8.1D). This transmission may occur directly, i.e., while the mother and the offspring have a physical connection (e.g., transovarial or transuterine), or indirectly, i.e., when mother and offspring remain close to each other after the birth. Mechanistically, the latter is a form of horizontal transmission because other susceptible hosts close to the mother could become infected as well.

Thus far, transmission from mother to offspring has been observed only in two parasites of *Daphnia*, both microsporidians infecting *D. magna* (*Flabelliforma magnivora* and *O. bayeri*). *O. bayeri* is also horizontally transmitted after the death of the host (Vizoso and Ebert 2004). For both parasites, it seems likely that transmission is transovarial.

As mentioned above, it is important to note that, mechanistically, horizontally transmitted parasites may appear to be vertically transmitted. If horizontally transmitted parasites can infect host offspring in the brood chamber or shortly after birth, they are functionally vertically transmitted. It is not clear how commonly this form of transmission occurs in *Daphnia*. The vertical transmission of parasites that are horizontally transmitted mechanistically is, however, common in other host–parasite systems (Ebert and Herre 1996).

A vertically transmitted parasite that has attracted a lot of attention for its high prevalence across arthropod taxa, including several crustaceans, is the intracellular bacterium Wolbachia. *Wolbachia* is transovarially transmitted and may be the most common parasite of arthropods worldwide. S. West and D. Ebert (unpublished observations) tested three clones from *D. magna* and three clones of *D. pulex* (each from a different population in southern UK) for the presence of either *Wolbachia* clade A or B (methods as in West et al. 1998). Although positive and negative controls confirmed that the PCR protocols worked properly, none of the *Daphnia* samples tested positive. The absence of *Wolbachia* was also reported by Fitzsimmons and Innes (2005), who tested *D. pulex* from the Great Lakes region of North America. Although the absence of evidence should not be taken as evidence for absence, I consider it highly unlikely that further investigations would reveal *Wolbachia* in *Daphnia*. Given our current knowledge of the mechanisms *Wolbachia* uses to maintaine itself in host populations (male killing, feminization, induced parthenogenesis, and cytoplasmic incompatibility), it seems unlikely that populations of cyclic parthenogens such as *Daphnia* could support *Wolbachia*.

8.1.2 Survival of Transmission Stages Outside the Host

An important factor for parasites with waterborne transmission is the lifetime of transmission stages outside of the host. The longer they can survive outside the host, the higher their likelihood of transmission. The longest surviving *Daphnia* parasites known thus far are the heavily protected endospores of the bacterium *P. ramosa*. In sediment cores of shallow ponds, spores more than 20 years of age have been found to be infectious (Decaestecker et al. 2004). Resting stages of *Daphnia* epibionts were even found to be viable after more than 60 years in the sediments (Decaestecker et al. 2004). Bacteria and microsporidian parasites can also be stored in freezers (-20°C) for several years without apparent loss of infectivity. Spores of the microsporidians *G. intestinalis* and *O. bayeri* survive for at least 6-12 months in dry conditions at room temperature (H.J. Carius, unpublished observations; D. Ebert, unpublished observations). *O. bayeri* survives summer droughts in rock-pool populations in southern Finland (S. Lass and D. Ebert, manuscript in preparation).

It seems plausible that parasites in aquatic systems face fewer problems surviving outside their hosts than their terrestrial counterparts, because the most common causes of transmission-stage mortality for air- and soilborne parasites do not exist for waterborne transmission stages. Desiccation, for example, is irrelevant in the aquatic environment. Furthermore, water not only provides protection from UV radiation to a large degree, but its high heat capacity also buffers the effects of rapid temperature changes and prevents overheating. Because it is costly to produce protective

structures for transmission stages (e.g., thick spore wall), aquatic parasites (as opposed to terrestrial parasites) may be able to shift the trade-off between quantity and quality of spores toward the production of more transmission stages.

8.1.3 Uptake of Transmission Stages from Pond Sediments

Planktonic populations typically undergo tremendous fluctuations in density, often over several orders of magnitude. Some plankton organisms might even temporarily disappear from their habitat and survive in the form of resting stages. Because these bottlenecks in host density pose a problem for horizontally transmitted parasites, Green (1974) suggested that plankton parasites should have persistent transmission stages to endure phases of low host density. He suggested that pond sediments form spore banks for these infective stages, similar to the way they harbor resting stages of many plankton organisms.

To test this hypothesis, mud samples were collected from different ponds that harbored parasitized populations of *D. magna*. Subsamples of these sediments were placed in beakers, and uninfected *D. magna* were added. When the hosts were later dissected, infections with different microparasites were found: among others, the bacterium *P. ramosa*, the yeast *Metschnikowia bicuspidata* , and the microsporidia *G. intestinalis* and *O. bayeri* (Ebert 1995; Decaestecker et al. 2002) (D. Ebert, unpublished observations). The results clearly confirm Green's (1974) hypothesis that pond sediments can serve as "parasite spore banks" and that parasites can survive periods of low host density in a "sit-and-wait" stage.

The uptake of spores from sediment is related in part to poor feeding conditions for the hosts and in part to their phototactic behavior. When feeding conditions deteriorate, some Cladocerans switch from filter feeding in the free water to browsing on bottom sediments. This behavior stirs up particles from the sediments, which are then ingested by filter feeding (Horton et al. 1979; Freyer 1991). What is important here is that spore uptake from pond sediments is primarily a density-independent form of transmission; it may only be linked to density indirectly, because high density may induce a switch in *Daphnia's* feeding behavior.

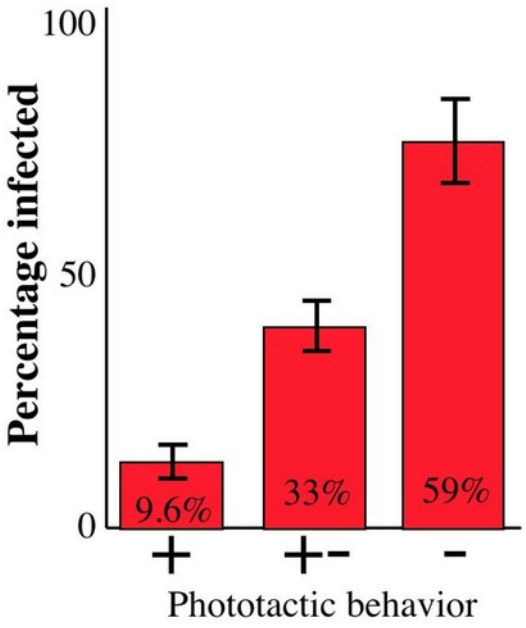

Figure 8.2 Relationship between the innate tendency of a *D. magna* clone to be positively (+), intermediately (+ -), or negatively (-) phototactic and the likelihood of contracting a disease from the pool sediment. Negatively phototactic clones tend to stay closer to the sediments and have a higher chance of picking up parasite spores from the sediments. Modified after Decaestecker et al. (2002).

There is also evidence that the phototactic behavior of *Daphnia* clones also affects their likelihood of catching sediment-borne diseases (Decaestecker et al. 2002). *D. magna* genotypes with negative phototactic behavior are much more likely to come in contact with pond sediments and thus catch a disease than clones with a positive phototactic behavior (Figure 8.2). Decaestecker et al. (2002) speculated that a trade-off between predator and parasite avoidance may be important in the evolution of habitatselection behavior. Negatively phototactic clones suffer less from visually hunting predators by residing in deeper and darker portions of the water column during the day, whereas positively phototactic clones, which are at a higher risk of predation, are less exposed to parasite spores in the sediment and consequently suffer less from parasitic infection. It was shown that increased

infection rates near the sediments can be triggered by changing the daphniids' phototactic behavior, exposing them to chemical cues from fish (kairomone) and thus inducing a general behavioral shift toward lower positions in the water. This trade-off highlights a cost of predator-induced changes in the *D. magna's* habitat selection behavior and may help to explain genetic polymorphism for habitat selection behavior and disease resistance in natural *Daphnia* populations (Decaestecker et al. 2002).

8.1.4 Factors Influencing Parasite Transmission

After a parasite appears in a host population, it can only survive if each infection causes on average at least one secondary infection, that is, the basic reproductive rate of the parasite, R_0, must be larger than 1 (Anderson and May 1986). There has been much discussion about what factors influence a parasite's transmission in a plankton population; I will summarize these below.

Parasite Transmission Is Density Dependent

Density-dependent transmission, which is a central assumption of much epidemiological theory for horizontally transmitted parasites, has often been discussed with regard to plankton parasites (Canter and Lund 1951, 1953; Miracle 1977; Brambilla 1983; Ebert 1995; Bittner et al. 2002). Convincing data for density-dependent transmission and host population regulation under natural conditions were presented by Canter and Lund (1953), who observed strong fluctuations of the diatom *Fragilariacrotonensis* in an English lake. Whenever the density of these planktonic algae reached more than about 100 cells/ml, a fungal parasite (*Rhizophidium fragilariae*) spread rapidly, and host density dropped by two orders of magnitude.

Density-dependent Transmission in Natural Populations For *Daphnia*, no such example exists, although published data do not contradict density dependence. Brambilla (1983) observed that a microsporidian was generally present whenever the *D. pulex* density rose above 10 animals/liter, although the parasite suddenly disappeared one year in mid-summer despite high host densities. Vidtmann (1993) observed that the microsporidium *Larssonia daphniae* was present only when *Daphnia* density was high and yet was often absent during periods of high host density. Similar results were reported by Yan and Larsson (1988). Ruttner-Kolisko (1977) described a significant relationship between the density of a rotifer and prevalence, and even attributed a strong population decline in *Conochilus unicornis* to a microsporidian epidemic: "... Plistophora finally terminates its host species". Stirnadel (1994) was not able to detect density-dependent interactions between any of three *Daphnia* species and their numerous microparasites. The same was observed by Decaestecker (2002) in a very similar study on *D. magna*. Despite this paucity of published evidence to prove that density dependence plays a critical role for *Daphnia* epidemiology, many studies note that there is a minimum host density for parasite persistence, although the behavior at high densities has yet to be determined. For the time being, experimental approaches are more helpful than observations for investigating the role of density-dependent transmission.

Experimental Evidence for Density-dependent Transmission The microspordian gut parasite*G. intestinalis* in *D. magna* has proved to be an ideal system to test for the density dependence of transmission. The life cycle of *G. intestinalis* is direct, and transmission to new hosts occurs only 3 days after infection (Ebert 1994a, 1995). The waterborne spores of this parasite are transmitted with the feces. Laboratory experiments showed that the transmission of *G. intestinalis* is strongly density dependent and that the infection intensity (parasite load per host) increased more rapidly when hosts were more crowded (Figure 8.3). Very similar experiments were conducted with the protozoan parasite *C. mesnili*, which infects *D. galeata* (Bittner et al. 2002). The higher the density, the more likely it was that *C. mesnili* was transmitted (Figure 8.3). These experiments were carried out by placing one infected and one uninfected host together in vials containing different volumes of medium. In smaller volumes, the likelihood of transmission was higher. Interestingly, however, the decline in transmission rate with increasing volume was much smaller than expected, assuming a dilution effect. A possible explanation for this

result is that two *Daphnia* within a vial do not distribute themselves randomly and independently from each other but rather cluster in certain parts of the vial, e.g., the bottom or places with more or less light. Therefore, on average, they are closer to each other than volume alone would suggest. Whether clustering plays a role in the transmission dynamics of natural populations is not known, but nonrandom distributions have frequently been observed in natural *Daphnia* populations (Green 1955; Weider 1984; Watt and Young 1992). Therefore, it appears likely to me that local clusters of *Daphnia* may play an important role in parasite dynamics in natural populations.

For parasites that are transmitted after the death of their host, density dependence has to be tested in a different way. Here it is the density of free transmission stages in the water that is important (Anderson and May 1986), and density-dependent transmission is indicated by infection–dose response curves. This has been shown for the yeast *M. bicuspidata*, the parasite *P. ramosa* (Ebert et al. 2000b; Regoes et al. 2003), and the microsporidium *O. bayeri* (Vizoso et al. 2005). In a very rigorous and detailed analysis, Regoes et al. (2003) showed that the likelihood of *P. ramosa* infecting *D. magna* largely followed the mass action assumption of classic epidemiology, which states that the likelihood of transmission is linearly related to the product of susceptible hosts and transmission stages (Figure 8.4).

Conclusions on Density-dependent Transmission From these experiments, one can conclude that density dependence is indeed a real phenomenon in the spread of horizontally transmitted parasitic infections in *Daphnia* populations. However, merely confirming that density-dependent transmission exists does not reveal its significance for epidemiology in natural populations. To date, little support has been found to verify that density dependence is an important factor in *Daphnia* parasite epidemics. Other factors that seem to play an important role in transmission may cloud the significance of density dependence. Among these factors may be the temperature dependence of transmission (Ebert 1995), host stress, the role of a spore bank in the sediments (Ebert et al. 1997), and the genetic structure of the host population with respect

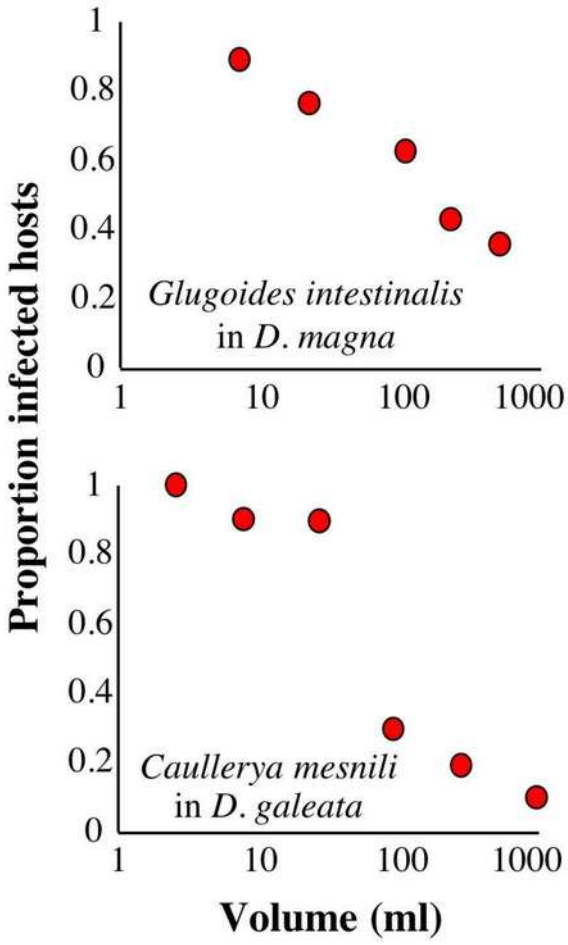

Figure 8.3 Relationship between culture volume and the likelihood of transmission of two gut parasites. *Top*, *G. intestinalis* in *D. magna*; *bottom*, *C. mesnili* in *D. galeata*. Redrawn after Ebert (1995) and Bittner et al. (2002).

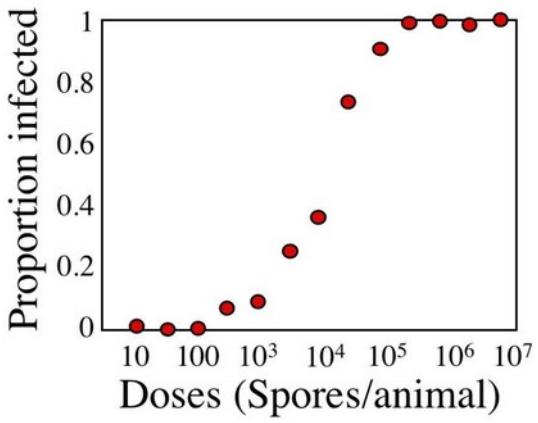

Figure 8.4 Proportion of infected *D. magna* relative to the number of *P. ramosa* spores in the medium. The sigmoidal increase in proportion of infected hosts follows the expectation of the mass action model closely. Each spore dose was replicated about 100 times. Redrawn after Regoes et al. (2003).

to susceptibility (Little and Ebert 2000; Carius et al. 2001).

Parasite Transmission Can Be Limited by Low Temperatures

Plankton epidemics are predominantly found during the warm summer months (Green 1974; Brambilla 1983; Yan and Larsson 1988; Vidtmann 1993). Ruttner-Kolisko (1977), working with a microsporidian parasite in a rotifer population, proposed that transmission is impaired at low temperatures. I tested this hypothesis with *G. intestinalis* in *D.magna* and found that transmission was indeed impaired below 12°C (Ebert 1995). This is consistent with the observation that *G. intestinalis* decreased in late autumn in *D. magna* populations in southern England (Stirnadel 1994). Poor transmissability at temperatures below 25°C was reported for *P. ramosa*, which parasitizes the Cladoceran *Moina rectirostris* (Sayre et al. 1979). (Note: It is questionable whether this *Moina* parasite was indeed *P. ramosa*.) In contrast, *P. ramosa* in *D. magna* can be transmitted between 10 and 25°C in the laboratory (Ebert et al. 1996; Mitchell et al. 2005). Thus, temperature criterion appears to be species and strain dependent.

Reports of natural *Daphnia* populations further indicate that certain parasites can be found under winter conditions (Stirnadel 1994; Bittner 2001). In Lake Constance, *Daphnia* parasites often occur predominately in fall and winter conditions (Bittner 2001), suggesting that temperature is certainly not universal in limiting parasite spread. The absence of parasites during summer in large lakes has been suggested to be related to intense predation during summer months (Duffy et al. 2005) and is unlikely to be a consequence of temperature effects on transmission.

Host Stress Might Facilitate Parasite Spread

It has been claimed that stressed host populations are more susceptible to parasites and thus facilitate epidemics. This theory has been used to explain disease outbreaks in Cladocerans kept under poor laboratory conditions (Seymour et al. 1984; Stazi et al. 1994). Likewise, France and Graham (1985) observed higher rates of microsporidiosis among stressed crayfish in acidified lakes. For *Daphnia*, there is no support for the stress hypothesis but rather the opposite. Experimental transmission of *G. intestinalis* to individual *D. magna* appeared to be largely independent of the host's feeding conditions (and did not differ among age groups or sex) (Ebert 1995). Similar results were obtained for *C. mesnili* in *D. galeata* (Bittner et al. 2002). A direct test of the stress hypothesis was carried out in experimental populations of *D. magna* infected with *G. intestinalis*. When half of the experimental populations were stressed (reduced food level), parasite populations suffered more than the host populations (Pulkkinen and Ebert 2004) because mortality was disproportionately higher among the most heavily infected hosts (those that carried the most parasites). This result counters conventional wisdom about vertebrate populations, in which stress is thought to go hand-in-hand with disease outbreak. Experiments that tested the relationship between transmission stage production and host nutritional status further support the observation that *Daphnia* parasites do not fare well when their hosts are stressed. As in other invertebrate systems, parasites in poorly fed hosts produce fewer transmission stages than parasites in well-fed hosts (Ebert et al. 1998). Thus, although some observations have been interpreted to suggest that stress may lead to disease outbreaks, experimental results show

clearly that this is not always the case, and this aspect of epidemiology needs further study.

Resistance May Limit the Spread of Diseases

It has been long known that host genotypes differ in their susceptibility to parasites, as has been shown for several combinations of *Daphnia* populations and parasite species (Ebert et al. 1998; Little and Ebert 1999; Little and Ebert 2000; Carius et al. 2001; Decaestecker et al. 2003). Furthermore, there is good evidence for strong host–clone x parasite (isolate and species) interactions, both within and across populations (Ebert 1994b; Ebert et al. 1998; Carius et al. 2001; Decaestecker et al. 2003) (Figures 5.2 and 8.5). These studies also reported local parasite adaptation, noting that local parasites were more aggressive (more infective, more virulent, higher growth rate) than novel, introduced parasites (Ebert 1994b; Ebert et al. 1998).

The strongest evidence that infections within a population depend on host genotype was found by Little and Ebert (2000), who showed that in 3 of 4 tested populations, female *D. magna* infected with *P. ramosa* under natural conditions were genetically more susceptible to this parasite. To test this observation, they took field samples to the laboratory, divided them into infected and uninfected females, cured them with an antibiotic, and then cloned and reinfected the hosts with *P. ramosa* from the same population. The clonal offspring of the formerly infected females needed lower spore doses to become reinfected than the offspring of the formerly uninfected females (Figure 8.6), thus indicating that genetic factors are clearly of crucial importance for the spread of diseases in natural *Daphnia* populations.

Summary of Transmission Limiting Factors

The four factors discussed above may represent only a few of the many that influence the spread of diseases in *Daphnia* populations; however, I believe that they represent the most important ones. Other factors may be specific to certain diseases or may play minor roles. Although none of the factors discussed is likely to play a key role throughout the growing season, one or a few of them may become more influential at certain phases in epidemics. Furthermore, factors may interact to counterbalance or re-enforce each other. Genetic variation for resistance may, for example, be deflated by host stress. Thus, to understand the factors that influence the spread and dynamics of diseases in natural populations, it is necessary to conduct experiments that disentangle the complex interactions of host–parasite interactions. Experimental epidemiology is a particularly promising approach for addressing these questions (see Chapter 7 on Experimental Epidemiology and Evolution of *Daphnia* Parasites).

8.2 Epidemiology of *Daphnia* Microparasites

The results discussed thus far indicate that the invasion, spread, and persistence of parasites in *Daphnia* populations cannot be attributed to a single factor. Rather, the relevant factors may vary over time and act together or against each other. This interplay shapes parasite dynamics. Although we do not currently have conclusive explanations for the seasonal dynamics of *Daphnia* parasites, what we do know can serve as a starting point for a better understanding of plankton epidemics.

8.2.1 The Fishless Pond Model

Most of what we know about *Daphnia* parasites comes from small, predominantly fishless water bodies. The epidemiology of most microparasites of pond-dwelling *Daphnia* in the temperate zone follows a similar pattern (Green 1974; Brambilla 1983; Vidtmann 1993; Decaestecker 2002). Prevalence is usually low in winter and early spring. After host densities peak in spring, parasite prevalence increases; it fluctuates throughout the summer and decreases in autumn, with parasites often disappearing completely in winter. Green (1974) suggested that some microparasite epidemics (e.g., the bacterium *Spirobacillus cienkowskii*) start when a benthic feeding host acquires a parasite from the mud. Once the cycle starts, other Cladocerans that are partially benthic and partially free-water foragers become infected and transmit the parasite to those Cladocerans that live in the free water. The parasites disappear from the pond when the hosts go into diapause at the end of the season.

Earlier I proposed a single species version of this model (in 1995; Ebert et al. 1997). Following

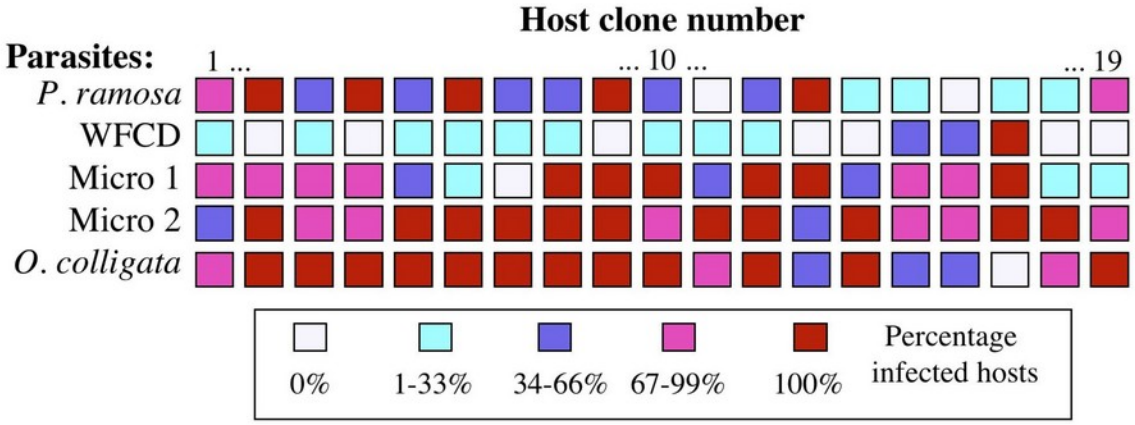

Figure 8.5 Variation in resistance among 19 *D. magna* clones in response to five parasite species. *Micro 1* and *2* are two undescribed microsporidian parasites of *D. magna*. All host clones and parasite isolates originated from the same population. Redrawn and modified after Decaestecker et al. (2003).

diapause, *Daphnia* hatch from their ephippia and recolonize a pond. Under good feeding conditions, the population increases rapidly during spring until food shortages lead to a switch from filter feeding in the free water to browsing on the bottom sediments. Browsing supplements the food because it stirs up food particles (Horton et al. 1979; Freyer 1991), which are then ingested by filter feeding. However, browsing also stirs up parasite transmission stages, which may infect the daphniid. Once the first hosts are infected, the disease may spread further. The epidemic ends either when environmental conditions deteriorate (e.g., low temperature) or when the host population becomes sparse or disappears altogether.

A key feature of this model is the uptake of spores from the pond sediments, which has very important consequences for the epidemiology of the system, as was shown in a mathematical version of this model (Ebert et al. 1997). First, uptake of spores from the sediments is independent from host density. The basic reproductive rate R_0 becomes redundant as a means of predicting parasite persistence when there is a large, nondepleting spore bank in the sediment. Instead, the feeding behavior of *Daphnia* and the properties of the resource determine parasite invasions. This may explain why longitudinal studies of *Daphnia* pond populations have failed to find a relationship between parasitism and host density. Second, the spore banks allow the parasites to survive long periods of low host density.

Although this epidemiological model was developed for pond dwelling zooplankton, its findings about density-independent infection could also be relevant to a number of soil-borne diseases. Fleming and colleagues (1986) investigated the density-dependent transmission of a virus in different populations of the soil-dwelling pasture pest *Wiscana* sp. (Lepidoptera: Hepialidae). Evidence for density-dependent transmission was found only in young pastures but not in old pastures, perhaps because in older pastures transmission occurred mainly from a spore pool that had accumulated over several generations. In laboratory populations of a virus–insect system, Sait and colleagues (Sait et al. 1994) failed to detect density dependence and attributed this result to the rapid accumulation and long persistence of virus transmission stages within the cages. Contamination of the soil has been repeatedly cited as the source of various infections (Kellen and Hoffmann 1987; Young 1990; Woods et al. 1991; Dai et al. 1996). Thus, it appears that durable transmission stages and their accumulation in pond sediments or soil might be a widespread phenomenon in natural host–parasite systems and may obscure any pattern of density-dependent host-to-host transmission.

The *Daphnia*–parasite model for fishless ponds offers only the most basic pattern of parasite dy-

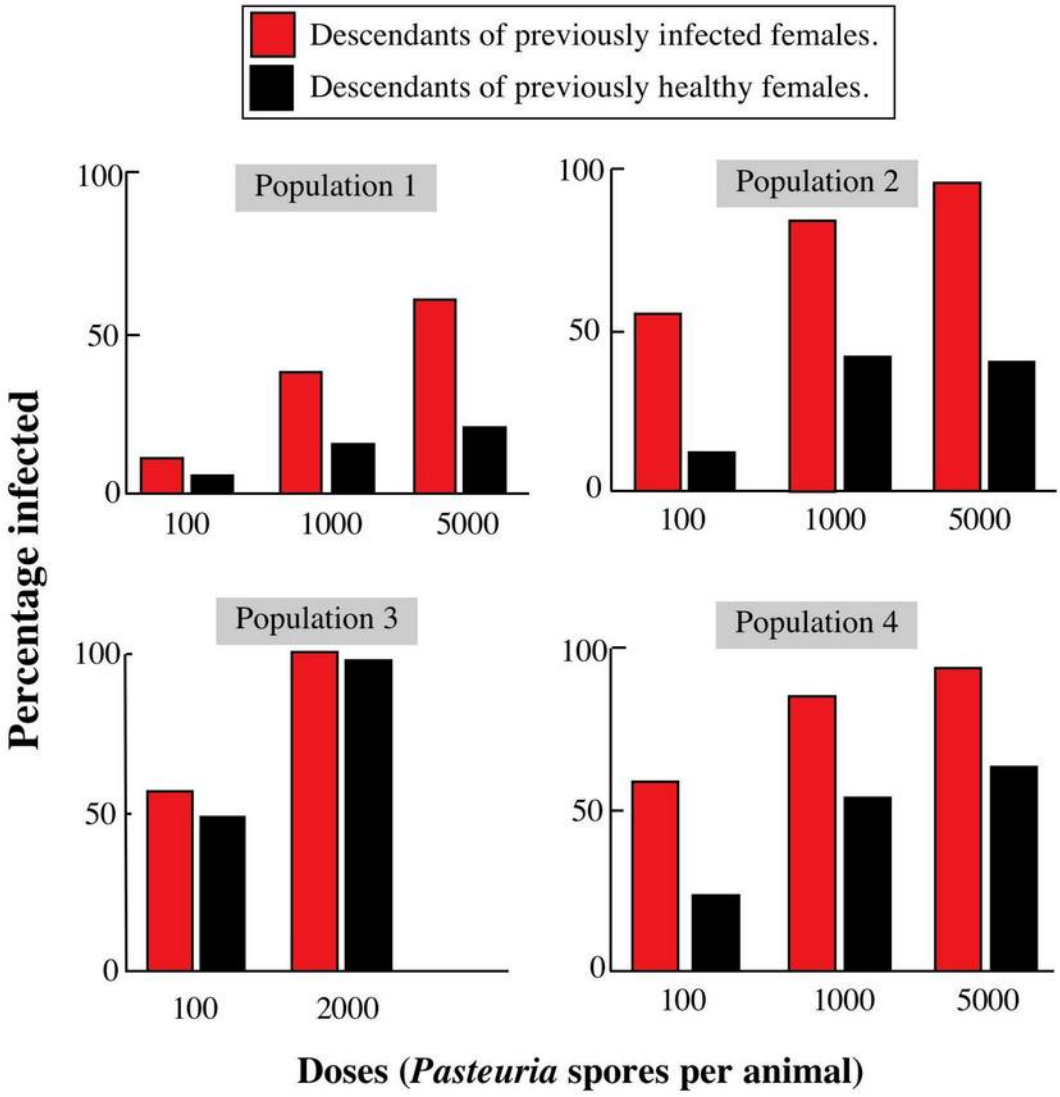

Figure 8.6 Difference in susceptibility of either infected or healthy *D. magna* collected from a natural population. All females were cured and reinfected under standardized conditions with different doses (spores of *P. ramosa* per host). In three of four populations, the descendants of previously infected females were more susceptible to infection under standardized laboratory conditions. Modified after Little & Ebert (2000).

namics, leaving many details unexplained. It cannot, for example, explain the dynamics of prevalence in lakes where there are likely to be no spore banks and may also fail to predict epidemics in ponds with permanent (without diapause) *Daphnia* populations. It is further unable to explain why certain parasite species show short-lasting epidemics of a few weeks. Clearly, our understanding of parasite dynamics in natural *Daphnia* populations is still very limited.

8.2.2 Suggestion for a Lake Model

As discussed above, lakes with fish predation seem to have lower rates of parasitism than fishless ponds (see Chapter 4 on *Daphnia* Microparasites in Natural Populations). The following model may be a starting point for understanding zooplankton epidemics in lakes with fish. My ideas are partially based on the work of Kerstin Bittner at Lake Constance (Bittner et al. 1998, 2002; Bittner 2001).

Fish predation can be a severe mortality factor for *Daphnia* and will certainly influence the abundance of parasites. If fish predation is high, parasites may not be able to spread in *Daphnia* populations, because the average life expectancy of a *Daphnia* (and thus of an infection) is too short (see Chapter 4, Are There Fewer Parasites in Lakes with Fish?). K. Pulkkinen and D. Ebert (manuscript in preparation) have shown high parasite extinction rates in artificially predated, experimental *D. galeata* populations. Thus, during periods of high predation, parasites are expected to be absent or found in low prevalence. Because predation pressure often varies over time, parasites may spread during periods when adult host mortality is relatively low. This theory coincides with findings that the prevalence of *Daphnia* parasites in lake populations is high in fall when fish predation is low, whereas parasites are absent or only found in low prevalence during summer time, when predation is high (Bittner et al. 2002; Duffy et al. 2005).

In fishless ponds, parasites survive the absence of their hosts in the sediments. Because lakes with fish are less likely to have ecologically important spore banks in the sediments (*Daphnia* are much less likely to come into contact with the sediment in lakes), a different hypothesis is needed to explain how these parasites can survive unfavorable conditions. A possible explanation might be the large size of plankton populations, which may enable parasites to survive long periods of negative population growth ($R_0 < 1$). With a huge host population size, for example, a parasite population might decline considerably for several generations, reaching very low prevalence. But low prevalence in large lakes is hardly an indication of extinction. For example, in a lake the size of Lake Constance (volume, 50×10^9 m^3), if the host density falls to 0.1 *Daphnia* per m^3 and 1 in 100,000 hosts is infected, there would be still about 50,000 infected hosts, certainly enough to maintain the parasite population, although at levels far too low to be detected with conventional sampling methods. This argument needs careful evaluation, taking absolute host and parasite population sizes into account as well as year-round growth conditions.

An alternative hypothesis is that parasites go extinct locally but occasionally recolonize the lake. However, if only one or a few immigrant parasites are introduced into a large host population, their spread to detectable levels takes considerable time unless R_0 is high ($\gg 1$). Nevertheless, this mechanism may still explain some of the observed cases of parasite disappearance and reappearance.

As mentioned above, parasites in large lakes with fish predation may evolve certain strategies to reduce their mortality. The most obvious of these are fast development (even if it has costs in terms of high virulence) and low visibility to visually hunting fish. A comparative study between lakes with and without fish predation would allow these two predictions to be tested.

In summary, parasites may be able to survive in large lakes with fish predation by exploiting hosts at times of low predation pressure and outlasting unfavorable times in a state of extended negative population growth.

8.3 Conclusions and Open Questions

At present, we have no satisfactory model for the epidemiology of *Daphnia* parasites, nor of any other zooplankton parasite. The two models presented above are general frameworks that treat all parasite species of a community alike and thus lack many important features. A more profitable approach may be to focus on certain parasite species

and attempt to understand their epidemiology. Research has shown unambiguously that although certain mechanisms work under controlled conditions, e.g., density-dependent transmission, they may not necessarily explain the relevant dynamics in the field. In my judgment, a combined laboratory and field research approach is needed to elucidate the epidemiology of parasites. It is not clear whether general principles will explain the dynamics of certain host–parasite interactions or whether biological details of the specific interaction are required to understand the most of the observed variance. Some milestones on the way may be the answers to these open questions:

1. Which factors limit the spread of microparasites in natural populations?
2. What role do spore banks in sediments play in natural systems?
3. Does density-dependent transmission explain parasite dynamics in natural populations?

Chapter 9

Population Dynamics and Community Ecology

Although much research has examined the effect of parasites on individual hosts, relatively little work has been done to address the impact of parasites on the host population, in particular on host population dynamics. Here I describe what is known about the impact of *Daphnia* parasites on host population density and persistence. A number of parasites have been shown to reduce host density and to reduce population persistence in experimental populations. Consistent with epidemiological models, the strength of these effects was highest for parasites that also have the strongest effect on reducing host fecundity. Thus far, little is known about the community ecological effects of parasites. The available data suggest, however, that parasites have the potential to influence competition among host species.

9.1 Background

Over the last decades, researchers have believed that freshwater zooplankton population dynamics were shaped by inter- and intraspecific competition and by predation. Only recently have parasites been recognized as a factor in the ecology and evolution of plankton communities. In their pioneering work, Canter and Lund (1951, 1953, 1968) showed that a fungal microparasite strongly altered the dominance hierarchy of a phytoplankton community in an English lake. Unfortunately, this work has not stimulated much research in the field. In particular, very little work has addressed the effect of parasites on zooplankton dynamics.

A number of studies using diverse host–parasite systems have shown that parasites can influence their host populations either by reducing host density or even by driving host populations to extinction (Park 1948; Finlayson 1949; Keymer 1981; Kohler and Wiley 1992; Hudson et al. 1998). These studies provide evidence that parasites can regulate their host populations and that some parasites are more likely to do so than others. Thus, one might also expect that zooplankton populations are regulated by their parasites. Ideally, one would like to predict which parasite features affect host population levels and under which conditions parasite effects are seen at the host population level. Several theories have been developed to understand whether variability in the effects of parasites on host fecundity and survival are reflected in host population dynamics (Anderson and May 1978; May and Anderson 1978; Anderson 1979; May and Anderson 1979; Anderson 1982; May and Anderson 1983; Anderson and May 1986; Anderson 1993). A key question is whether processes at the individual level translate to effects at the population level. We have good empirical data on processes at the individual level (e.g., pathogenicity) for a number of host–parasite systems but little on population-level processes.

Mathematical models predict different population dynamics for hosts infected with microparasites that reduce host fecundity *versus* those infected with parasites that reduce host survival (Anderson 1979, 1982). Host density is predicted to decrease monotonically, with the negative effect that a parasite has on host fecundity (all other things being equal). In contrast, mean host population density is predicted to first decrease and then increase as parasite-induced host mortality rises. This is because (for a given transmission rate parameter) parasites that kill their hosts very rapidly are less likely to be transmitted to other hosts and will, therefore, remain at low prevalence, whereas parasites with little effect on host mortality will have little effect on host demographics. These epidemiological models also predict population fluctuations, positing that host density fluctuations increase as a microparasite shows an increasingly negative effect on host survival and fecundity. According to these models, density fluctuations increase the chance of extinction of small host populations because host density is more likely to drop to zero during population bottlenecks (May 1974; McCallum and Dobson 1995). Epidemiological models, such as those cited above, have often been used to explain empirical results in situations where parasites reduced the density of their hosts or contributed to the extinction of the host population. The same models predict that benign parasites have little effect on host population densities and therefore can be applied equally well to cases where parasites have little or no apparent effect on host population dynamics. Therefore, along with contrasting parasitized with nonparasitized populations, it is important to compare host populations infected by parasites with different effects on host fecundity and survival.

9.2 Do Parasites Regulate Host Populations?

A review of field studies on parasitism in *Daphnia* populations (see Chapter 4, Generalizations about Parasitism in Natural Populations) reveals very little about the population-level effects of parasites on their hosts. Because there are no replicates or control populations without parasites in field studies, it is difficult to draw conclusions about population-level effects. To my knowledge, only Brambilla (1983) has attempted to analyze his data for possible population-level effects of parasitism. He tested for the effect of the microsporidium *Thelohania* on the instantaneous birth and death rates in a longitudinal study of a *D. pulex* population and compared these rates with rates calculated under the assumption that the parasite was absent from the population. The impact of the parasite on birth rate varied widely over the summer and across the year but was generally stronger than it was for the death rate. For nearly all sampling dates, he calculated that the parasites decreased the population growth rate, r, by about 20% on average. He states, however, that the parasite alone probably does not regulate the population growth of its host, because r varied substantially, independent of parasitism (Brambilla 1983). He was not able to carry out laboratory experiments.

Population-level experiments with *Daphnia* parasites were first proposed by Ebert and Mangin (1995), who showed that *D. magna* populations infected with the microsporidium *Flabelliforma magnivora* (in their paper called *Tuzetia* sp.) had a lower density than uninfected control populations. This parasite is exclusively vertically transmitted under laboratory conditions (horizontal transmission has not been found for this parasite) and was present at a prevalence of 100%. Therefore, one can exclude density-dependent transmission as the regulatory factor. Because exclusively vertically transmitted parasites in asexual populations behave like a deleterious gene (Mangin et al. 1995), the reduced density is a direct consequence of the reduced fecundity and survival of the hosts.

Ebert et al. (2000a) compared the effects of six parasites on the fecundity and survival of individual hosts to their effects on host population density and the host's risk of extinction. Five horizontally transmitted microparasites (two bacteria: White Fat Cell bacterium, *Pasteuria ramosa*; two microsporidia: *Glugoides intestinalis, Ordospora colligata*; one fungus: *Metschnikowia bicuspidata*) and six strains of a vertically transmitted microsporidium (*F. magnivora*) of *D. magna* were used. Life table experiments quantified fecundity and survival in individual parasitized and healthy hosts and compared these with the effect of the parasites on host population density and on the likelihood of host population extinction in microcosm populations.

Parasite species varied widely in their effects on host fecundity, host survival, host density reduction, and the frequency with which they drove host populations to extinction (Figure 9.1). The fewer offspring an infected host produced, the lower the density of its population. This effect on host density was relatively stronger for vertically transmitted parasite strains than for the horizontally transmitted parasites. There was no clear relationship between the reduction in host density and the effect of parasites on the survival of individual hosts. As predicted by stochastic simulations of an epidemiological model, if a parasite had strong effects on individual host survival and fecundity, the risk of host population extinction was also increased. The same was true for parasite extinctions.

Bittner et al. (2002) showed that the gut parasite *Caullerya mesnili* is not only able to reduce density in experimental *D. galeata* cultures severely but also that it is able to drive the host population to extinction. This result is consistent with the study by Ebert et al. (2000a), which showed that *C. mesnili* is highly virulent, reducing host fecundity strongly and shortening the host's life span substantially. This parasite was also able to alter the outcome of competition among two competing *Daphnia* species. In the absence of the parasite, *D. hyalina* was inferior to *D. galeata*, whereas in its presence, *D. hyalina* was the superior competitor (Bittner 2001).

In a 27-week time series study of *Glugoides intestinalis*-infected *D. magna* cultures, Pulkkinen and Ebert (2004) found no significant reduction in host density, nor did they record a single case of host or parasite extinction. Again, these results are consistent with the predictions and results of Ebert et al. (2000), because *G. intestinalis* is comparatively avirulent, reducing host fecundity by only about 20% and barely influencing host survival.

In summary, parasites in experimental *Daphnia* populations have been shown to reduce host density and population survival. In particular, as the theory predicts (Anderson 1982; Ebert et al. 2000), parasites with strong effects on host fecundity are powerful agents for host population regulation. Thus far, all experiments have been conducted under laboratory conditions, i.e., with constant food supply, constant temperature, absence of predators, etc., so that the populations closely reflected an idealized host–parasite system, as many standard epidemiological models envision (Anderson 1979, 1982; Ebert et al. 2000). However, although these experiments have helped us understand the mechanisms of host–parasite epidemiology, they have not answered the question of whether parasites regulate natural *Daphnia* populations, a question that may require experimental epidemiology under more natural conditions (e.g., mesocosm populations).

9.3 Do Parasites Influence Host Community Structure?

Thus far, we have discussed the impact of parasites on single host species. As a further step, one might ask whether parasites can influence entire host communities. Two characteristics of parasites place them in a prime role to affect community ecology. First, they are often specific in the effect on their hosts, and second, they may exert strong harm on their hosts, influencing the host's competitive ability. A few data suggest that parasites of *Daphnia* may indeed play a role in the structure of their host's community.

Wolinska et al. (2004) studied parasitism in a pre-alpine lake (Greifensee) in Switzerland. In this lake, *D. galeata* x *hyalina* hybrids co-occur with the parental taxa. Interestingly, during the study period, hybrids were the most abundant taxon. The *Daphnia* community in this lake is parasitized by *C.mesnili*, which is known to be rather virulent (Bittner et al. 2002). Prevalence reached peaks of 22%, and *C. mesnili* dramatically reduced *Daphnia* fecundity. A comparison among the different taxa revealed that hybrids were frequently infected, whereas parental *D. galeata* (the other parent species, *D. hyalina*, was rare during the study period) were almost never infected. The authors speculate that the resistance of *D. galeata* might counterbalance the greater fitness of hybrids. This could stabilize the coexistence of the parental species with the hybrids in Lake Greifensee. It is not clear whether the high susceptibility of the hybrids is a general phenomenon or specific to this population. In any case, the finding adds an important aspect to the puzzling question of hybrid maintenance in natural *Daphnia* populations and hints at a role of parasites in shaping *Daphnia* communities.

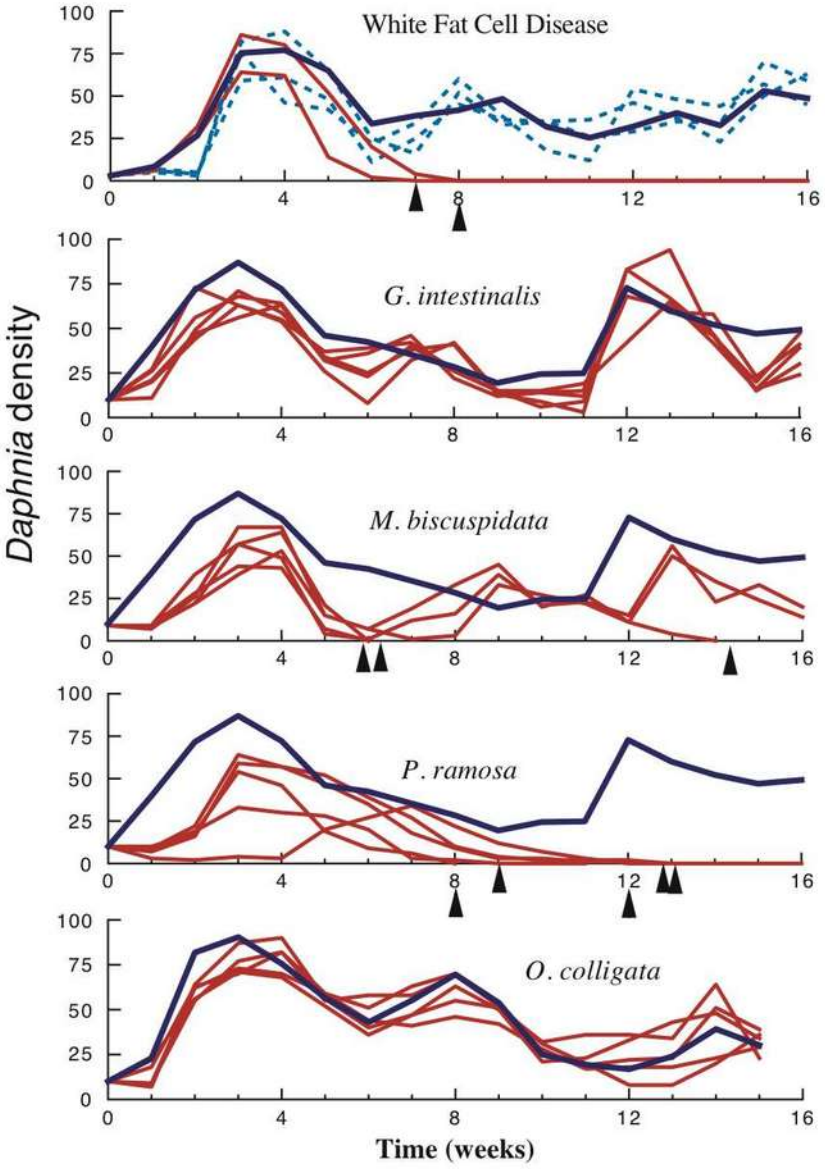

Figure 9.1 Host density in relation to parasitism. The five graphs show *D. magna* density changes in population experiments with five horizontally transmitted parasites (five replicates per treatment). The *thick dark blue line* shows the mean of the host densities of the five control populations. The *light blue stippled line* shows replicates in which the parasite became extinct. Note that they follow the control lines. The *red lines* show the replicates of the parasitized populations. Note that the avirulent gut microsporidians *G. intestinalis* and *O. colligata* show little effect on host density. In none of these replicates did the host become extinct. The other three parasites reduce host density by variable degrees and bring the host population to extinction (indicated by *black arrowheads*) in some cases. Redrawn and modified after Ebert et al. (2000).

Bittner (2001) took an experimental approach to study the role of *C. mesnili* in a two-species community of *D. galeata* and *D. hyalina* in Lake Constance. To test whether this parasite, which frequently parasitizes both species, influences their relative competitive ability, Bittner set up a number of population-level experiments in which clones of both *Daphnia* species competed in the presence and absence of the parasite. Clones were tracked with the help of multi-locus enzyme electrophoresis, and the experiments resulted in a very clear pattern. In the presence of *C. mesnili*, *D. hyalina* was the superior competitor, whereas it was inferior in its absence. This finding was consistent across several clones of both species. Of interest, *D. hyalina* is not completely resistant to the parasite but seems to suffer much less under the costs of parasitism. Bittner's results (2001) show clearly that parasites do have the potential to alter competition in a plankton community. However, although the experiments convincingly demonstrate the mechanism, they do not provide us with a way to judge the importance of this mechanism in natural communities.

In summary, because of their differential effects on different host taxa, parasites have the potential to influence competition in *Daphnia* communities, much in the same way as they influence clonal competition within a species (Capaul and Ebert 2003; Haag and Ebert 2004) (Figure 6.2). We know little about the strength of this mechanism under natural conditions and about the role of predation in this phenomenon. A combined approach with experimental and observational work in the field may help to clarify the role of parasites in shaping *Daphnia* communities.

9.4 Factors Structuring Parasite Communities

In several places in this book, I have discussed that parasiteabundance may be negatively influenced by other natural enemies of *Daphnia*, in particular by planktivorous fish. See the sections "Are There Less Parasites in Lakes with Fish?" in Chapter 4 and "Suggestion for a Lake Model" in Chapter 8 for more details. Predation by visually hunting fish would not only suppress certain parasites species during particular time periods, or completely (Duffy et al. 2005), but would also influence the parasite community by disfavoring parasite species that make their hosts more susceptible to predation, for example, by making their hosts more visible. Although we are starting to understand the dynamics between fish and certain parasites, we do not know anything about the community-level consequences of this relationship.

Another factor that affects parasite communities is interspecific competition. Because hosts are limited resources, within-host competition may be intense and may influence the success of a species on the community level, particularly among parasites with ecologically similar niches (Kuris and Lafferty 1994; Lafferty et al. 1994; Poulin 1998). The best evidence for interspecific competition comes from epibionts rather than endoparasites. Competition was favored as an explanation for the presence/absence patterns of epibionts in two rock-pool metacommunity studies in southern Finland (Green 1957; Ebert et al. 2001). The peritrich *Vorticella octava* was found to be negatively associated across rock pools with the peritrich *Epistylis helenae* and the green algae *Colacium vesiculosum*. All three species primarily colonize the head and dorsal regions of the *Daphnia* carapace. However, *V. octava* was found together with *E. helenae* and *C. vesiculosum* much less often than chance would suggest, whereas *Epistylis* and *C. vesiculosum* occurred independently of each other. This may occur because of the different space requirements of these epibionts on the host's body surface. *Colacium* has a short stalk, whereas *V. octava* and *E. helenae* have long stalks and may form a canopy over *Colacium*. Moreover, *E. helenae* has a noncontractile stalk, whereas *V. octava* has a contractile stalk that, when it contracts, forms a spiral larger than the diameter of the stalk. This contraction may cause a mechanical disturbance to both *Colacium* and *Epistylis* and lead to stronger competition (Green 1957). Thus, *V. octava* may suffer from strong interspecific competition because it interferes mechanically with both *E. helenae* and *C. vesiculosum*, whereas the two latter species do not compete as strongly with each other because they are somewhat separated in space.

Earlier, Green (1955) had shown experimentally that peritrichs (species not given) compete with *C. vesiculosum* and that light is an important factor in determining the outcome of competition between algal epibionts (favored under strong light) and

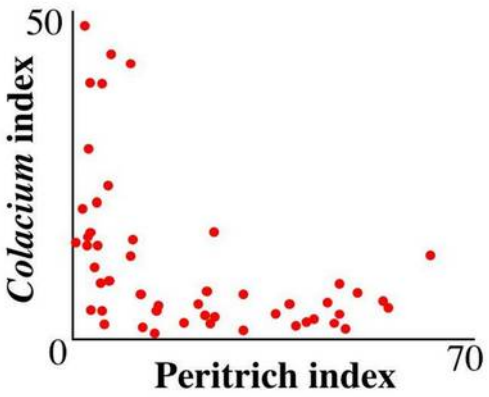

Figure 9.2 Relation between the densities of *Colacium vesiculosum* and peritrichs on *D. magna*. These data are collected from a natural population. In experiments it was shown that light favors *C. vesiculosum* over peritrichs, whereas darkness favors peritrichs over *C. vesiculosum*. This indicates that the negative correlation in the abundance of these two epibionts is driven by interspecific competition. Redrawn from Green (1955).

peritrich ciliates (favored under poor light conditions). Across several individuals within a population, this competition leads to a negative correlation between the number of peritrichs and the number of *C. vesiculosum* (Figure 9.2). The strong variation in epibiont composition across individuals may reflect individual differences in behavior. For example, clones with a phototactic-positive behavior may have more algae than phototactic-negative clones.

These findings clearly demonstrate the strength of within-host competition for shaping entire metapopulation communities. The clearness of the patterns is surprising, however, given that similar strong patterns are rarely seen from other parasites. I speculate that a combination of specific host–epibiont interaction factors play a role here. First, *Daphnia* molt every few days (1-2 days as juveniles and 3-4 days as adults at 20°C). After molting, the carapace is clean, and epibionts struggle to recolonize it (Threlkeld et al. 1993). Thus, competition for space is reset after every molt, strongly diminishing the role of history (who colonizes first) and leading to stronger homogenization among hosts in the entire population. Second, the low virulence (harm done to the host) caused by epibionts decouples host mortality from the action of epibionts. Third, there is likely to be little or no immune defense of the host against epibionts. All of these factors are different for endoparasites, which are unaffected by host molting but are affected by the immune response of the host and may be virulent for the host. To my knowledge, no study has yet demonstrated parasite competition in plankton hosts.

9.5 Conclusions and Open Questions

It seems rather clear that parasites have the potential to influence host population dynamics and communities and that interspecific competition and ecological factors affecting the host influence parasite communities. What we are lacking are general patterns that would allow us to make predictions for systems we have not yet studied. For this, we need to study not one species or one community at a time but several in parallel. A number of issues have not yet been addressed regarding plankton parasites. Here I suggest a few questions for further research:

1. Some parasites may alter the outcome of host competition. Which properties of a parasite affect host competition, and which do not?
2. Is there interspecific competition among endoparasites in plankton hosts?
3. Are there trade-offs between competition at different levels? For example, a parasite might be a good competitor on a host but is poor in dispersal among hosts or among populations.
4. Do evolutionary processes (e.g., clonal selection) influence community aspects?

Chapter 10

Experiments with *Daphnia* and Parasites

This chapter describes how to use the *Daphnia* parasite system for experiments. I first discuss the advantages of the system for research and education. Then I describe a number of experiments, some of which are very simple and are suitable for courses in experimental parasitology and ecology. The experiment section has two parts. The first discusses experiments in which the individual host is the unit of replication. Such experiments can be used to ask questions such as: How does a parasite affect its host? How is a parasite transmitted? The next part expands to discuss experiments that use entire populations as the unit of replication. Here I suggest experiments that pose questions such as: Does a parasite influence host density? Can a parasite drive its host population to extinction? How quickly can hosts evolve resistance?

10.1 Advantages of Using the *Daphnia*–Parasite System for Experiments

The *Daphnia*–parasite system is particularly suitable for testing hypotheses because it allows for the creation of rather simple experiments. Among the advantages of this system are:

- Under laboratory conditions (20°C), *Daphnia* produce their first eggs after 7-15 days (depending on the food level). This equals the shortest possible generation time in experiments. Thereafter, they produce a clutch of parthenogenetic eggs every 3-4 days until death, which results in an approximately constant fecundity across the adult life span. The first clutch is usually smaller than the following clutches. Only very low food levels may result in skipped clutches.
- Controlled conditions allow other extrinsic sources of mortality, e.g., predation by fish, infection by other parasites, to be excluded.
- Parthenogenetic reproduction allows the females to remain isolated (1 female in 30-200 ml of culture medium) so that fecundity and death schedules can be recorded accurately. From these, birth and death rates can be calculated in the absence of density dependence. Individual females can be kept with or without parasites.
- Parthenogenetic reproduction further allows for the separation of genetic (among-clone variance components) and nongenetic effects (within-clone variance components).
- Many *Daphnia* parasites fit the definition that epidemiological models use for microparasites very well: small, unicellular parasites that reproduce directly within their hosts and are

directly transmitted among hosts (Anderson and May 1991).

- Many parasites allow for the freezing of transmission stages, which means that the same genetic material can be stored for long time periods. *Daphnia* can be kept clonally for very long times (years!) and thus can be kept essentially without genetic changes for long periods.

For experiments with replicated populations (rather than individuals), the following points are also relevant:

- The population growth of *Daphnia* in laboratory populations with a constant food supply and no parasites is reasonably well described by a logistic growth model. In the absence of parasites, *Daphnia* populations reach an equilibrium population level that represents carrying capacity. *Daphnia* populations have overlapping generations. Generation times in population experiments are about 10 to 15 days (at 20°C).

- Clonal reproduction of hosts avoids complications attributable to mate choice and mate finding. It also excludes complications attributable to the effects of inbreeding or outbreeding.

- *Daphnia's* planktonic way of life approximates well-mixed conditions without strong spatial structure. Transmission of most *Daphnia* parasites is through waterborne transmission stages. These follow the common epidemiological assumption of mass action nicely, which states that the likelihood of transmission is strictly a function of the population densities (or sizes) of infected and uninfected hosts.

- Epidemiological models usually assume homogeneously mixed populations without genetic structure. Monoclonal *Daphnia* populations fulfill these criteria perfectly. This is advantageous for an experimental system because the absence of genetic host diversity allows one to exclude the confounding effect of host evolution, which may otherwise rapidly change the genetic structure of the populations (Capaul and Ebert 2003). In monoclonal *Daphnia* populations, genetic diversity can only arise by mutations, and mutation rates are too low to play a significant role in experimental *Daphnia* populations that are kept for a limited period (less than a few years).

- *Daphnia* parasites usually produce persistent (chronic) infections. Unless the host clears the infection within the first 1 or 2 days after exposure, it will not recover from the infection. Therefore, only two classes of hosts need to be considered in epidemiological models—infected and uninfected hosts. This simplification is very helpful for understanding the epidemiology.

- Polymorphic allozyme markers are available for nearly all *Daphnia* species, allowing one to identify multi-locus genotypes very cheaply and quickly (Hebert and Beaton 1993). With an established routine, it is possible to type more than 1000 individuals on a working day, enabling one to follow clone frequencies in replicated populations and monitor microevolutionary changes.

10.2 Using Proper Controls: Placebos

Because experimental studies must compare the treatment group with the control group, the control group must be handled the same way as the treatment group in every respect, except for the experimental factor (parasites, for most purposes in the context of this book).

Here I want to mention a few points about the use and preparation of placebos. A placebo refers to a control treatment that resembles the other treatment in all aspects except the one that is being tested. There are two crucial points to consider when using placebos.

First, the placebo has to resemble the treatment in all factors except the actual treatment factor. Thus, when preparing a spore suspension with macerated tissue from infected *Daphnia*, the placebo must be prepared with tissue from uninfected *Daphnia*. Using water as a placebo is not enough, because it differs in more than the absence

of transmission stages from the treatment suspension. It may also lack nutritional material for the *Daphnia* that the treatment suspension may contain.

Second, the placebo may have some effect on the controls. This effect, often called a placebo effect, describes a difference between the placebo treatment and a totally untreated control. If the effect of the actual treatment and the effect of the placebo treatment do not influence each other, this is not a problem, but if the effect of the placebo interacts with the effect of the actual treatment, the results may be difficult to interpret. For example, suppose you test for the immune response of a host after it is exposed to parasitespores. If both the placebo and spore suspensions contain compounds that influence the immune response of the host (e.g., certain bacteria), one obtains estimates of host response, which have to be seen within the light of this suspension. A water control may not have the same effect. The response to the exposure to spores may have been different if the spores had been in a water suspension without any other compounds. I recommend, therefore, using two controls in individual-level experiments: a placebo control and a control without anything. You may not be able to avoid a placebo effect, but it is important to know about it.

10.2.1 Uninfected Controls in Parasite Studies

Controls have more functions than just being the sample against which the treatment is tested. When testing for the effect of certain treatments on a parasite's performance, infected hosts should be kept under different treatment conditions (e.g., parasite growth under different environmental conditions; transmission rates under different densities). Because all treatment groups are infected, an uninfected control does not seem necessary. There are reasons why uninfected controls (actually placebo-exposed controls) should be included. First, the uninfected controls allow you to verify that all material was uninfected before the start of the experiment. Second, some experiments fail for unknown reasons, e.g., there may be high unexplained mortality. The controls allow you to judge whether the parasites played a role in these results.

10.2.2 Using Additional Treatments as a Quality Control

In certain experiments, it is not clear whether the treatment applied will show any effect. A negative result is difficult to present in a convincing way, because the nonsignificance of the treatments may have been caused by other reasons than the absence of an effect—the absence of evidence is not evidence for absence. For example, statistical noise may disguise a treatment effect in a poorly executed experiment. To ascertain the quality of the experiment, I recommend using an additional factor that is known to produce a visible effect, even if this effect is not the focus of your research question. For example, one may use two food levels, along with the other treatment. Then if a food effect is apparent, you may convince the observer that other treatment effects could also be found, provided they are there. If you fail to find a food effect, your experiment may have been poorly performed.

10.3 Experiments with Individuals

A number of *Daphnia* parasites can easily be bred under laboratory conditions and are therefore suitable for experimental work. These experiments can be conducted in courses on the evolution and ecology of host–parasite interactions but also for research purposes. What follows are some suggestions for simple experiments that will work even if one has little experience with *Daphnia* parasites.

10.3.1 Effects of Exposure Dose on Parasite and Host Success

The transmission stages of horizontally transmitted parasites may be administered to the host in different concentrations. Typically, higher doses are more likely to produce infections (Ebert et al. 2000b; Regoes et al. 2003). To quantify the infection success of parasite isolates, a standardized measure is used: the ID50 (or infective dose 50%), which is the dose at which 50% of the exposed hosts become infected. The ID_{50} may vary strongly among parasite isolates and host clones (Ebert 1998b). It is usually estimated with a statistical procedure

based on infection data (binary data) in response to several different dose treatments.

The success of a parasite also depends on its within-host growth, which in turn depends on within-host competition. The more transmission stages that enter a host, the more competition will occur, thus lowering the success of each individual parasite. In extreme cases (very strong competition resulting from very high doses), the parasite may completely fail to conclude its development (Ebert et al. 2000b). High doses of the parasite may also harm the host more strongly. It has been observed that, with increasing spore dose, host mortality and morbidity increases (Ebert et al. 2000b).

Dose experiments can easily be done with every parasite that is transmitted from a dead host. Transmission stages are collected from dead hosts, and suspensions are produced with different concentrations of spores. Spore concentrations may be varied over several orders of magnitude to observe clear-cut effects. Parasites that are transmitted from living hosts may also be used in dose experiments. For these experiments, one exposes the recipient host to different-sized groups of infected hosts (Ebert 1995). I suggest using at least 10 replicates per dose level to facilitate the statistical analysis.

10.3.2 Testing for Mode of Transmission

It is often a challenge to determine the mode of transmission for unknown parasite species. For a course in ecological and evolutionary parasitology, it can be a rewarding exercise to run a series of experiments with selected parasites to determine their mode of transmission. The experiments to test for mode of transmission can be extrapolated from the chapter on transmission. Keep in mind that some parasites can transmit using more than one mode.

10.3.3 Estimating the Harm Done to the Host

Conventionally, one thinks of a parasite as detrimental to the host. However, because it is often difficult to test for the effect of the parasite on its host, there is some belief that many parasites are not harmful. Here I suggest testing for the effect of parasites on the survival and fecundity of individual females. For the simplest type of experiment, a split brood design is useful. In this design, females must be kept under very good conditions so that they produce large clutches of offspring. Females around 15-25 days old produce the largest clutches. Shortly after their release from the brood chamber, offspring should be isolated in individual jars; half of them should be exposed to the parasite, the other half to a placebo. Animals need to be fed daily, and medium must be changed every 3-4 days. The individuals of both treatment groups should be checked daily for survival and offspring production. Detailed descriptions of similar experiments have been published (Ebert 1995; Ebert and Mangin 1997; Bittner et al. 2002).

10.4 Experimental Epidemiology and Evolution of *Daphnia* Parasites

An alternative to experiments on individuals is to investigate the effect of parasites on their host populations. Such experiments allow the investigator to ask questions that cannot be answered on the individual level, such as: Do infected populations have lower population densities than parasite-free populations? Can parasites drive their host populations to extinction? Do infected populations have more pronounced population size fluctuations? Do hosts/parasites show an evolutionary response to their antagonist?

The beauty of experiments on the population level is that the results relate more closely to the processes in natural systems because they include interactions that arise from the fluctuating numbers of community members, e.g., effects of density-dependent population growth, density dependence of transmission processes, and effects of genetic and demographic (age and size) population structure.

Daphnia and its microparasites compose one of the few systems where both host and parasites have generation times short enough to allow experimental ecological and evolutionary studies to be carried out in real time. The wide range of parasites available allows for the testing and compar-

ison of epidemiological, evolutionary, and genetic models of infectious diseases.

The basic outline of such experiments is rather simple. One can start populations from stock cultures with a mixed age and size distribution. Populations should be large enough to minimize random effects, such as genetic drift, chance extinctions, or large unexplained variation among replicates. Once treatment groups are formed and treatments applied, populations can be followed in regular intervals over long periods of time. It is important to think ahead about the way in which the populations are sampled, because sampling itself may introduce some effect.

The use of experimental epidemiology and evolution as research tools is still not very widespread. Here I introduce a few studies that use these methods in the hope of stimulating more experimental approaches of this type.

10.4.1 Host Starvation and Parasite Load in Experimental Populations

Outbreaks of epidemics in vertebrate populations have often been linked to host stress. No similar predictions have been made about the response of invertebrates to stressful conditions. A population-level experiment was designed to test for the effect of food stress on the epidemiology of the gut parasite *Glugoides intestinalis* (Pulkkinen and Ebert 2004). Infected and uninfected *D. magna* populations, which had been kept for many generations under a constant high food supply, were exposed to a severe reduction in the amount of available food. Infected and uninfected control populations continued to receive the full amount of food. Changes in parasite and host population size as well as host body length were recorded to determine how the food shortage influenced host and parasite population dynamics. In both infected and uninfected populations, food shortage led to an approximately equal reduction in host density and changes in host body length distribution. Large hosts suffered from higher mortality than smaller hosts, which significantly reduced the mean body length in the starved populations. Because this change was stronger in the infected populations and because large hosts usually carry the most parasites, this change led to a reduction of average parasite spore load and prevalence in the starved populations. These results indicate that food stress for hosts impairs parasite spread in this system and that host mortality can be an important factor in regulating parasite abundance at the population level.

10.4.2 Parasitism and Host Competitive Ability

Parasites may influence the competitive ability of their host. This effect can be pronounced when parasites show some degree of specific virulence for otherwise superior competitors. A simple experiment to investigate this effect is to set up populations with two *Daphnia* species and follow their populations in the presence and absence of a parasite. Bittner (2001) conducted such an experiment with competition between *D. galeata* and *D. hyalina* and the parasite *Caullerya mesnili*. In the presence of *C. mesnili*, *D. hyalina* was the superior competitor, whereas it was inferior in the absence of *C. mesnili*.

Because parasites may alter the competitive ability of certain clones, similar experiments may be done with competition among clones of one *Daphnia* species. Capaul and Ebert (2003) allowed 21 clones of *D. magna* to compete in the presence or absence of different parasite species in 10-liter aquaria with a population size of about 1000 animals. The outcome of clonal competition was not only very rapid (strong changes were evident after only 2 months) but also differed among all treatments. A similar design was chosen by Haag (2004), who allowed clones of *D. magna* to compete under outdoor conditions in mesocosms (rain tons). He also found strong changes in clonal composition that were dependent on time and parasite treatment.

10.4.3 The Experimental Evolution of Virulence

Population-level experiments may also be used to study the evolution of parasites. To test for the effect of host demography on the evolution of parasite virulence, a laboratory experiment was set up in which parasites were allowed to evolve. If the life expectancy of a parasite is short, it is expected to evolve at a higher rate of host exploitation and, therefore, higher virulence, because its

penalty for killing the host is minimized. This hypothesis was tested by keeping the horizontally transmitted microsporidian parasite *G. intestinalis* in monoclonal cultures of *D. magna* under conditions of high and low host background mortality (Ebert and Mangin 1997; Ebert 1998b). High host mortality and, thus, parasite mortality, was achieved by replacing 70-80% of all hosts in a culture with uninfected hosts from stock cultures every week (replacement lines). In the low mortality treatment, no replacement took place. Contrary to expectations, parasites from the replacement lines evolved a lower within-host growth rate and virulence than parasites from the nonreplacement lines. Across lines, a strong positive correlation between within-host growth rate and virulence was found. The unexpected result was explained by the more severe within-cell competition in the nonreplacement lines, which may have led to selection for accelerated within-host growth. These results point out that single-factor explanations for the evolution of virulence can lead to wrong predictions and that multiple infections are an important factor in virulence evolution.

10.5 Conclusions and Open Questions

The *Daphnia*–parasite system has proved to be a wonderful system for experimental and observational studies, both on the individual and the population levels. In my 15 years of research with this system, I found only two aspects of this system to be lacking, which would make it even more powerful:

1. Genetic markers for parasites. This would allow us to study parasite evolution more directly.

2. Breeding parasites on artificial medium, i.e., outside the *Daphnia* host.

Glossary

This glossary was prepared with the help of the following sources: Allaby (1994), Decaestecker (2002), Dobson and Grenfell (1995), Freeman and Herron (2001), Isaacs et al. (1991), King and Stansfield (1997), and Margolis et al. (1982).

Abdominal processes Processes on the abdomen of *Daphnia* that close the brood chamber.

Abundance How commonly a taxon or group of taxons occurs. Usually used without units. More precise terms are distribution, prevalence, and density.

Adaptation 1. Process by which populations undergo modification so as to function better than their immediate ancestors in a given environment. 2. Any developmental, behavioral, anatomical, or physiological characteristic of an organism that improves its chances for survival and propagation in its environment. See also Local adaptation.

Additive genetic variance Part of the phenotypic variance of quantitative traits, such as body size or age at maturity. The additive genetic variance is proportional to the expected change attributable to selection and is used to calculate the heritability.

Allele One of a series of possible alternative DNA sequences at a given locus.

Allozyme Gene product of one of several alleles that have the same function but differ in their amino acid sequence and therefore in their physio-chemical properties so that they migrate different distances in an electrophoretic assay. They are used as genetic markers to identify a genotype.

Apomixis Form of asexual reproduction. Offspring is formed without meiosis and fertilization. Daughters are genetically identical to their mothers.

Arms race Occurs when an adaptation in one species reduces the fitness of individuals in another species, thereby selecting in favor of counter-adaptations in the other species. These counter-adaptations, in turn, select in favor of new adaptations in the first species. Arms races are a form of antagonistic coevolution. See also Coevolution.

Branchiopoda See Phyllopoda.

Brood chamber Space between the thorax and the dorsal carapace of Cladocera in which the oviduct ends and the eggs develop. It is in direct contact with the exterior medium.

Carapace Hard shell of crustaceans.

Cecum (caecum; plural, ceca; intestinal or hepatic or digestive caecum) One of the pair of small appendages of the Daphnia midgut. They are sealed from the gut by a membrane and may participate in the production of digestive fluids.

Cladocera Order of the Entomostraca. They have a bivalve shell covering the body but not the head, four to six pairs of legs, and two pairs of antennae used for swimming. They mostly inhabit fresh water. See also Entomostraca.

Clone Group of organisms that have arisen from a single female by asexual reproduction and are therefore genetically identical. A clone is often called an iso-female line.

Coevolution Changes in the genotypes of two or more species that are a direct consequence of

the species' interaction with one another. Coevolution can occur among mutualists and host–parasite pairs, as well as among entire groups of interacting organisms (e.g., pollinator–plant systems).

Crustacea Aquatic arthropods characterized by the presence of biramous appendages and two sets of antennae. Examples include crabs, lobsters, copepods, barnacles, shrimps, and waterfleas.

Cyclical parthenogenesis Mode of reproduction in which phases of parthenogenetic (asexual) and sexual reproduction alternate. Several asexual generations may follow a sexual generation. Found in Cladocera, Rotifera, and aphids.

Cyclomorphosis Seasonal change in phenotype of many plankton species. For example, some *Daphnia* species produce spines to protect themselves against predators during the summer season.

Deme Population that is sufficiently isolated so that it can be considered an evolving unit. Deme is more typically used by evolutionary biologists.

Density dependence Indicates that the intensity of a process depends on the density of a population. When fecundity or individual survival in a population are negatively dependent on density (e.g., parasite-induced host mortality), the process could potentially regulate population density. Transmission of horizontally transmitted parasites is usually host density dependent.

Depth selection behavior Behavior by which the zooplankton maintains a particular vertical distribution in relation to the stratification of the water (light, temperature, food, predation pressure). See also DVM.

Diapause Resting period during unfavorable conditions, e.g., during winter freezing or during draughts.

Diel vertical migration (DVM) Special case of depth selection behavior in which the preferred depth changes in a diel (daily) pattern.

Dose effect A change in response to exposure to some agent attributable to a change in that agent's concentration. For example, the increase in virulence or infection risk for hosts during exposure to increasing parasite spore doses.

Electrophoresis Method to study the movement of charged molecules in solution in an electrical field. The solution is generally held in a porous support medium such as cellulose acetate or a gel made of starch, agar, or polyacrylamide. Electrophoresis is generally used to separate molecules from a mixture based upon differences in net electrical charge and also by size or geometry of the molecules, dependent upon the characteristics of the gel matrix.

Endemic Permanent presence of a parasite population in a host population. Compare Epidemic.

Endoparasite Symbionts located within the body of the host. They may be intra- or extracellular.

Ephippium (plural ephippia) 1. Membranous external walls surrounding the resting eggs (usually sexual eggs) of Cladocera. 2. Resting stage of Cladocera consisting of one or two resting eggs, surrounded by a membranous external wall.

Epibiont Organism that lives attached to the body surface of another organism. Sometimes regarded as ecto-parasites. In zooplankton, epibionts are often ciliates, algae, bacteria, and fungi.

Epidemic Sudden, rapid spread or increase in the prevalence or intensity of an infection. Compare Endemic.

Epidemiology Study of infectious diseases and disease-causing agents on the population level in a parasitological context. It seeks to characterize the disease's patterns of distribution and prevalence and the factors responsible for these patterns. In a more applied context, it also strives to identify and test prevention and treatment measures.

Evolution Changes in allele frequencies over time.

Experimental epidemiology Study of epidemiology in replicated experimental populations.

Experimental evolution Study of evolutionary change in replicated experimental populations.

Fitness Extent to which an individual contributes its genes to future generations in relation to the contribution of other genotypes in the same population at the same time.

Genetic polymorphism Occurrence of two or more genotypes in a population.

Genetic variation Degree to which members of a population differ at certain loci.

Genotype Genetic composition of an organism as distinguished from its physical appearance (phenotype).

Gigantism Phenomenon describing increased growth (or large body size) of certain members of a population. Sometimes parasitized hosts show gigantism compared with nonparasitized conspecifics. In this case, gigantism is often associated with parasite-induced host castration.

Habitat The living place of a population, characterized by its physical, chemical, and/or biotic properties.

Helminth Wormy parasite. Helminths are not a taxonomic group.

Horizontal transmission Parasite transmission between infected and susceptible individuals or between disease vectors and susceptibles.

ID_{50} See Infective dose 50%.

Induced defense Defense that is only expressed in response to a specific stimulus.

Infection intensity 1. Number of parasite individuals in an infected host individual. 2. Mean number of parasites within infected members of the host population.

Infective dose 50% Number of parasite transmission stages (exposure doses) that results in 50% of hosts being infected.

Instar Discrete stages of development in insects and crustaceans, whose growth is accomplished by molting.

Kairomone Chemical cues released from predators and recognized by the prey. Kairomones from several different predators have been reported to lead to adaptive morphological and life history changes in *Daphnia*.

Local adaptation Genetic differentiation attributable to selective forces specific to the local environment. Local adaptation is best demonstrated by showing that immigrant genotypes are inferior to resident genotypes. Locally adapted parasites usually show higher levels of damage and have higher levels of transmission stage production in their local hosts.

Macroparasite Parasite that usually does not multiply within its definitive hosts but instead produces transmission stages (eggs and larvae) that pass into the external environment or to vectors. Macroparasites are typically parasitic helminths and arthropods. The key epidemiological measurement is generally the number of parasites per host.

Mass action Concept used to describe the transmission dynamics of infectious diseases. Mass action transmission occurs at a rate directly proportional to the number or density of both susceptible individuals and infected individuals in the population.

Maxillary gland See Shell gland.

Melanin Substance used by invertebrates to (among other functions) encapsulate parasites. See proPO system.

Metapopulation Group of partially isolated populations belonging to the same species. Migration among subpopulations is important for the ecological and evolutionary dynamics of a metapopulation.

Microparasite Parasite that undergoes direct multiplication within its definitive hosts (e.g., viruses, bacteria, fungi, and protozoa). Microparasites are characterized by small size

and short generation times. The key epidemiological variable, by contrast with macroparasites, is whether the individual host is infected.

Microsatellite locus Place in the genome where a short string of nucleotides, usually two to five bases long, is repeated in tandem. The number of repeats at any given locus is usually highly variable (many alleles) in a population and can be used for DNA fingerprinting.

Morbidity State of ill-health produced by a disease. Includes aspects of reduced fecundity, lethargy, and other signs of disease.

Multiple infections Infection in which an individual is infected by parasites of more than one species or more than one genotype of the same species.

Parasite richness See Richness.

Parasite 1. Disease-causing organism. 2. Organism exhibiting an obligatory, detrimental dependence on another organism (its host). Conceptually, parasite and pathogen are the same. Endoparasites live in the host's interior (They may be intra- or extracellular). Ectoparasites live on the surface of the host.

Parthenogenesis Development of an organism from an unfertilized egg. See also cyclic parthenogenesis.

Pathogen Disease-causing microorganism, such as viruses, bacteria, and protozoa. In the context of this book, equivalent to parasite.

Phenotypic plasticity Phenotypic variation expressed by a single genotype in different environments.

Phototactic behavior Behavior that is expressed in the presence of light stimuli.

Phyllopoda Order of Entomostraca including a large number of species, most of which live in fresh water. They have flattened or leaf-like legs, often very numerous, which they use for swimming. Also called Branchiopoda.

Population dynamics Changes in the population size through time. Also used to describe change in the demographic structure of the population (sex ratio, age and size structure, etc.).

Population Group of interbreeding individuals and their offspring. In asexual species, this definition cannot be applied; in this case, a population is a group of phenotypically matching individuals living in the same area.

Population growth rate (Malthusian growth rate, *r*) Measure of population growth. The instantaneous rate of increase of a population or genotype. It is used as a measure of fitness.

Predator-induced defense Defense reaction of prey triggered by the presence or action of a predator so as to reduce the expected damage of the predator.

Predator An animal that kills its victim, the prey item, and then feeds on it to subsist until the next kill.

Prepatent phase In helminth infections, time period from infection until a female starts to produce eggs. It is equivalent to the latent period in microparasitic infections.

Prevalence Proportion of host individuals infected with a particular parasite. Often expressed as a percentage. A measure of how widespread an infection or disease in a host population is. Sometimes used to indicate the proportion of infected hosts in a sample with any parasite species. In many studies, prevalence is measured only in a certain fraction of hosts. In zooplankton studies, often only adult hosts or adult females are considered. Prevalence is usually underestimated in field samples because new infections may escape detection by the investigator.

Primipare Female producing offspring or eggs for the first time.

proPO system (prophenol-oxidase system) The proPO activating system plays several functions in invertebrate immunity and is considered one of the most important defense mechanisms. The oxireductase phenoloxidase (PO) is part of a complex system of proteinases, pattern recognition proteins, and proteinase inhibitors that constitute the proPO activating

system. It is thought to be part of the invertebrate's immune response against parasites because the conversion of proPO to active enzyme can be initiated by molecules from invading microorganisms. PO is the final enzyme in the melanization cascade, which is a common response to parasite entry in many invertebrates. During a successful immune reaction, melanin encapsulates the invader and kills it.

Red Queen hypothesis Hypothesis that states that the adaptive importance of genetic recombination is to create genetic variation among the offspring, which is important in confrontation with coevolving parasites.

Resistance Reduction in host susceptibility to infection.

Resting egg See Ephippium.

Richness Number of parasite species per host individual or the mean number of parasite species within members of the host population.

Selection Process by which certain phenotypes are favored over other phenotypes. Selection leads to adaptation. Clonal selection is found when clones differ in their lifetime reproductive success and is usually seen in the form of genotype frequency changes.

Sex allocation Allocation of resources into male and female functions. For *Daphnia*, which reproduce asexually for most of the life cycle and thus produce mostly daughters, sex allocation refers to the extent to which males and resting eggs are produced.

Shell gland Organ found in *Daphnia* that may have a role in excretion and/or osmoregulation.

Sit-and-wait Strategy of parasites and predators to come in contact with their host or prey. It relies on the antagonist being active, while the parasite or the predator is waiting motionless. Many parasite transmission stages can endure long time periods before they are activated by an encounter with the host.

Specificity Describes the observation that only a subset of hosts is susceptible to infection. A high specificity refers to the observation that only a few host lines can be infected by a given parasite.

Spore In a parasitological context, transmission stage.

Spore bank Spores resting in soil or sediments.

Spore load Number of spores or sporophorous vesicles of a parasite (e.g., microsporidium, bacterial) in a host individual. It is a measure of parasite infection intensity and may be used to calculate parasite multiplication rate within the host.

Susceptible Accessible to or liable to infection by a particular parasite.

Symbiont Organism living together with another organism. This includes mutualists, parasites, and commensals.

Trade-off Unescapable compromise between one trait and another. In evolutionary biology, it is important because a negative genetic correlation between two traits, both of which affect fitness, limits their response to selection (a fitness-increasing change in one trait is coupled with a fitness-decreasing change in the associated trait).

Transmission The process by which a parasite passes from a source of infection to a new host. Horizontal transmission is transmission by direct contact between infected and susceptible individuals or between disease vectors and susceptible individuals. Vertical transmission occurs when a parent conveys an infection to its unborn offspring, as in HIV in humans.

Transmission stage Life stage of a parasite that is able to cause a new infection.

Vertical migration See Diel vertical migration.

Vertical transmission Parasite transmission from parent to offspring.

Virulence Morbidity and mortality of a host that is caused by parasites and pathogens. More specifically, it is the fitness component of the

parasite that is associated with the harm done to the host.

Wolbachia Intracellular bacteria that commonly infect a variety of arthropod species and induce various changes in its hosts' life history, sex allocation, and sex ratio.

Zooplankton Animal component of small aquatic organisms that mainly drift with water movements. They include protozoans, small crustaceans, and in early summer, the larval stages of many larger organisms.

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